

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C12Q 1/68, A62D 3/00</b>		A2	(11) International Publication Number: <b>WO 00/63443</b> (43) International Publication Date: 26 October 2000 (26.10.00)
(21) International Application Number: PCT/US00/09883 (22) International Filing Date: 13 April 2000 (13.04.00)		(81) Designated States: CA, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 60/129,511 15 April 1999 (15.04.99) US		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): HENDRICKSON, Edwin, R. [US/US]; 49 Kings Grant Road, Hockessin, DE 19707 (US). EBERSOLE, Richard, C. [US/US]; 2412 Dacia Drive, Wilmington, DE 19810 (US).			
(74) Agent: FELTHAM, S., Neil; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).			
(54) Title: NUCLEIC ACID FRAGMENTS FOR THE IDENTIFICATION OF DECHLORINATING BACTERIA			
(57) Abstract <p>A unique 16S rRNA profile derived from <i>Dehalococcoides ethenogenes</i> has been identified and isolated. The profile contains a nucleic acid fragment that is linked to dechlorinating activity. This sequence is set forth in SEQ ID NO:1.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

TITLE

NUCLEIC ACID FRAGMENTS FOR THE IDENTIFICATION  
OF DECHLORINATING BACTERIA  
FIELD OF THE INVENTION

5       The invention relates to the field of molecular biology and microbiology. More specifically, 16S rRNA regions of been identified and isolated from *Dehalococcoides ethenogenes* that enable the identification of dechlorinating bacterial strains. Probes and primers corresponding to the unique regions have been constructed to enable the rapid identification of the dechlorinators.

10      BACKGROUND  
Groundwater pollution by halogenated, and particularly chlorinated solvents is a worldwide problem associated primarily with industrial sites where mishandling or improper disposal has brought these solvents in contact with the soil. The most common and problematic compounds are the chlorinated ethylenes (ethenes) such as tetra- tri- or di-chloroethylene. Carbon tetrachloride, chloroform and methylene chloride are also pervasive pollutants. The reasons for concern are basically threefold. First, most of these solvents are sparingly soluble in water and have the tendency to stick to soil particles. This results in tenacious underground plumes of solvent which cannot readily be removed by standard pump and treat technology (Biswas, N., et al., *Water Environ. Res.* 64, 170, 10, 1 (1992); Hutter, G. M., et. al., *Water Environ. Res.* 64, 69, (1992)). Second, the toxicology of many chlorinated solvents suggests that these compounds may be carcinogenic and damaging to specific organs such as the liver and kidneys (Price, P. S., Memo of the U.S. Environmental Protection Agency, Office of Water, Washington, D.C.(1985); Vogel, T. M., *Environ. Sci. Technol.*, 21, 722, (1987)). Finally, under conditions found in many aquifers and subsurface environments, chlorinated ethylenes and methanes are very slow to be degraded biologically. The result of these factors is that chlorinated solvents are long-lived potentially hazardous groundwater pollutants.

30      Currently there are two approaches to in situ removal of organohalogen pollutants. The first approach is the standard "pump and treat" method where groundwater is pumped to the surface for physical stripping of the contaminant from the water. For chlorinated solvents this is more of a containment method than a remediation technology although given sufficient time (typically decades to centuries) this method may capture most of the pollutant. The other approach is biological in nature and utilizes microorganisms for the enzymatic transformation of the halogenated organics. The biological approach may utilize microorganisms indigenous to a particular site where the remediation process consists primarily of

making additions to the contaminated site that enhance the growth of the desired microorganism. Alternatively, nonindigenous microorganisms may be introduced to a contaminated site with the necessary amendments needed for growth.

A number of organisms are known to dechlorinate persistent chlorinated  
5 pollutants. For example, *Dehalobacter restrictus* and *Dehalospirillum multivorans*, have been shown to partially dechlorinate chlorinated ethenes (Kochian et al., *Plant Mol. Biol.* 46:237 (1995); Delhaize et al., *Plant Physiol.* 107:315 (1995)). Similarly, *Dehalococcoides ethenogenes* has been shown to effect the complete dechlorination of tetrachloroethene and trichloroethene to  
10 ethene [Freedman et al., *Appl. Environ. Microbiol.* 55:2144 (1989)] and Maymó-Gatell et al. (*Science*, 176:1568 (1997)) have isolated a *D. ethenogenes* strain that is capable of respiratory reductive dechlorination of tetrachloroethene directly to ethene with hydrogen as an electron donor. Analysis of the 16S rRNA  
15 of the Maymó-Gatell organism revealed a unique profile that may be used to identify organisms of similar reductive capabilities.

The first step in utilizing the dechlorinating properties of the above identified organisms is rapid and accurate identification. One method of identification involves the use of DNA probes (see for example in WO 89/06704, U.S. Patent No. 4,851,330, and U.S. Patent No. 5,574,145). Many such probes  
20 derive from the observation (see Woese, *Scientific American* 244 (6) 1981 for review) that parts of the 16S and 23s ribosomal RNA (rRNA) sequences vary in different species. This information was used initially for phylogenetic analyses but it has more recently been used for DNA probe-based methods for the  
identification of organisms. The utility of such a method is based on the  
25 conservation of nucleic acid sequence within the rRNA sequences.

Each of the cells of all life forms, except viruses, contain ribosomes and therefore ribosomal RNA. A ribosome contains three separate single strand RNA molecules, namely, a large molecule, a medium sized molecule, and a small molecule. The two larger rRNA molecules vary in size in different organisms.  
30 Ribosomal RNA is a direct gene product and is coded for by the rRNA gene. This DNA sequence is used as a template to synthesize rRNA molecules. A separate gene exists for each of the ribosomal RNA subunits. Multiple rRNA genes exist in most organisms, many higher organisms containing both nuclear and mitochondrial rRNA genes. Numerous ribosomes are present in all cells of all life  
35 forms. About 85-90 percent of the total RNA in a typical cell is rRNA. A bacteria such as *E. coli* contains about  $10^4$  ribosomes per cell. Much of the sequences in rRNA highly conserved across broad evolutionary boundaries,

however, certain regions are highly variable and may be used to make fine distinctions between species, sub-species and strains (U.S. Patent No. 5567587).

The problem to be overcome therefore is to identify a unique 16S rDNA sequence in a bacteria capable of dechlorination of persistent chlorinated compounds for the identification and ultimate enhancement of that bacteria to remediate a contaminated site. Applicants have solved the state problem by providing a set of nucleic acid sequences that are unique to various strains of *Dehalococcoides ethenogenes*.

#### SUMMARY OF THE INVENTION

The present invention provides an isolated 16S rDNA sequence indicative of a dechlorinating bacterial strain selected from the group consisting of: (a) SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:30; (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS at 65 °C; and (c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

The invention further provides primers useful for the identification of new dechlorinating bacteria selected from the group consisting of: SEQ ID NOs:9-29; and any sequences that hybridize under conditions of 0.1X SSC, 0.1% SDS at 65 °C to those primers.

The invention additionally provides an isolated bacterial strain comprising any one of the sequences of the instant invention as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:30, wherein said strain has the ability to dechlorinate chlorinated compounds.

The invention further provides a method for identifying a dechlorinating bacterial strain comprising: (i) extracting genomic DNA from a cell suspected of being able to dechlorinate chlorinated compounds; (ii) probing the extracted genomic DNA with a probe derived from any one of the sequences instant invention as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:30, under suitable hybridization conditions, wherein the identification of a hybridizable nucleic acid fragment

confirms the presence of a bacteria capable of dechlorinating chlorinated compounds.

Similarly the invention provides a method for identifying a dechlorinating bacterial strain comprising (i) extracting genomic DNA from a cell suspected of being able to dechlorinate chlorinated compounds; and (ii) amplifying the extracted genomic DNA with an oligonucleotide primer corresponding to a portion of any one of the sequences instant invention as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:30, such that amplification products are generated wherein the presence of amplification products confirms the presence of a dechlorinating bacterial strain.

The invention additionally provides a method for the dechlorination of chlorinated compounds comprising contacting a chlorinated compound with an isolated bacterial strain comprising any one of the DNA fragments as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:30 under conditions suitable for dechlorination to occur.

BRIEF DESCRIPTION OF THE  
DRAWINGS AND SEQUENCE LISTING

Figure 1 is an alignment of 16S rDNA sequence profile from *Dehalococcoides ethenogenes* DHE-195 as disclosed in Maymó-Gatell et al., *Science*, 276:1568 (1997), as compared with profiles generated for organisms isolated from a number of wastewater treatment sites.

Figure 2 is a comparison of the instant dechlorinating 16S rDNA profiles with a 16S rDNA profile from *E. coli*.

Figure 3 is a graph illustrating the ability of a soil microcosm or culture developed from certain soils taken from a chloroethene contaminated site to dechlorinate trichloroethylene or perchloroethylene.

Figure 4 is an image of an electrophoresis gel used to detect PCR products in a test of soils contaminated with chloroethenes using two sets of the primers described herein.

The following sequence descriptions and sequences listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825. The

Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are 5 herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

SEQ ID NO:1 is a unique region of the *Dehalococcoides ethenogenes* 16S rDNA profile which is linked to dechlorinating activity.

SEQ ID NO:2 is the 16S rDNA profile of *Dehalococcoides ethenogenes* 10 DHE-PL, isolated from soil surrounding in industrial site.

SEQ ID NO:3 is the 16S rDNA profile of *Dehalococcoides ethenogenes* DHE-STF, isolated from soil surrounding in industrial site.

SEQ ID NO:4 is the 16S rDNA profile of *Dehalococcoides ethenogenes* DHE-DAB, isolated from soil surrounding in industrial site.

15 SEQ ID NO:5 is the 16S rDNA profile of *Dehalococcoides ethenogenes* DHE-PIN, isolated from soil surrounding in industrial site.

SEQ ID NO:6 is the 16S rDNA profile of *Dehalococcoides ethenogenes* DHE-DLL, isolated from soil surrounding in industrial site.

20 SEQ ID NO:7 is the 16S rDNA profile of *Dehalococcoides ethenogenes* DHE-195 as reported in Maymó-Gatell et al. (*Science*, 176:1568 (1997)), Genbank AF004928.

SEQ ID NO:8 is the consensus sequence derived from DHE-PL, DHE-STF, DHE-DAB, DHE-PIN, and DHE-DLL at bases E180-E226.

25 SEQ ID NO:9-29 are primers derived from the 16S rDNA profile, useful in the identification of dechlorinating bacteria.

SEQ ID NO:30 is the consensus sequence derived from DHE-PL, DHE-STF, DHE-DAB, DHE-PIN, and DHE-DLL at bases E1001-E1047.

SEQ ID NO:31 is the base sequence in the region of the consensus 16S rDNA profile from where the diagnostic sequence is derived.

30 SEQ ID NO:32 is the base sequence in the region of the DHE-195 16S rDNA profile from where the diagnostic sequence is derived.

SEQ ID NO:33 is the *E. coli* reference 16S rDNA sequence.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides unique 16S rDNA sequence profiles 35 derived from *Dehalococcoides ethenogenes* (DHE). *D. ethenogenes* is known for its ability to degrade persistent chlorinated pollutants. The instant sequence profiles may be used to identify and sub-type bacteria with similar metabolic pathways. One sequence (ATTTTCTAGCGAGACTGCCCGCG, SEQ ID

NO:1), beginning at base E1146, has been identified in all DHE's isolated from contaminated soils and is strongly linked to the ability of these organisms to degrade chlorinated organics. Similarly, a stretch of nucleic acids ranging between E180 and E226, corresponding to SEQ ID NO:8 may be used to identify dechlorinators as well as for genetic sub-typing of species.

5 In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

The term "*Dehalococcoides ethenogenes*" will be abbreviated "DHE".

10 The term "DHE-195" will refer to the strains of *Dehalococcoides ethenogenes* isolated and characterized by Maymó-Gatell et al. (*Science*, 176:1568 (1997)).

The terms "DHE-PL, DHE-STF, DHE-DAB, DHE-DLL and DHE-PIN" will refer to strains of *Dehalococcoides sp.* containing the instant dechlorinating 16S rDNA profile.

15 The term "dechlorinating bacteria" refers to any bacterial species or strain that has the ability to remove at least one chlorine atom from a chlorinated organic compound. Dechlorinating bacteria may have the ability to grow on chlorinated organics as a sole carbon source, or may prefer degradation using an alternate energy source.

20 The term "chlorinated compounds" will mean any straight chain or ring containing organic compound which contains at least one chlorine atom.

Trichloroethylene will be abbreviated "TCE".

Perchloroethylene will be abbreviated "PCE".

25 The term "16S rDNA" will refer to the DNA encoding ribosomal RNA found within bacterial cells.

The term "16S rDNA profile" will refer to the specific DNA sequence of the rDNA gene in any particular organism. For the purposes of the present invention the 16S rDNA profiles for DHE-195, DHE-PL, DHE-STF, DHE-DAB, DHE-DLL and DHE-PIN are illustrated in Figures 1 and 2.

30 The term "signature sequence" or signature sequence region" will refer to those short sequences in the 16S gene or rRNA molecule which are unique to a certain group or groups of organisms. These sequences can be used to define domains, group, subdivisions genera or species of an organism.

35 The term "consensus sequence" as used herein, as it relates to the alignment of a given set of sequences, will be defined as the sequence of the set of bases where a designated base is the one that occurs most often at each position in the 16S sequence.

The term "reference sequence" as used herein, as it relates to the alignment of a given set of sequences, will be defined as the particular 16S sequence to which the bases at each position of an alignment of 16S sequences are compared. The reference sequence used herein was an *E. coli* 16S rDNA sequence. Bases identified in the reference sequence that correlate to corresponding bases in a 16S rDNA profiled are assigned an "E number". Thus, the base labeled E-27 on the reference sequence corresponds to base 1 of the 16S rDNA profile of DHE-195 and E-107 corresponds to base 66 of DHE-195. The complete correlation is given in Table 2.

10 The term "dechlorinating 16S rDNA profile" will refer to a 16S rDNA profile containing the diagnostic sequence as set forth in SEQ ID NO:1.

The term "diagnostic sequence" will refer to the sequence ATTTTCTAGCGAGACTGCCCGCG (SEQ ID NO:1) which is indicative of dechlorinating activity.

15 The letters "A", "G", "T", "C" when referred to in the context of nucleic acids will mean the purine bases Adenine (C5H5N5), Guanine (C5H5N5O) and the pyrimidine bases Thymine (C5H6N2O2) and Cytosine (C4H5N3O) respectively.

20 In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

25 The term "nucleic acid fragment" will refer to a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

30 The term "oligonucleotide" refers to primers, probes, oligomer fragments to be detected, labeled-replication blocking probes, oligomer controls, and shall be generic to polydeoxyribonucleotides (containing 2-deoxy-D-ribose), to polyribonucleotides (containing D-ribose) and to any polynucleotide which is an N glycoside of a purine or pyrimidine base (nucleotide), or modified purine or pyrimidine base. Also included in the definition of "oligonucleotide" are nucleic acid analogs (e.g., peptide nucleic acids) and those that have been structurally modified (e.g., phosphorothioate linkages). There is no intended distinction between the length of a "nucleic acid", "polynucleotide" or an "oligonucleotide".

35 The term "primer" refers to an oligonucleotide (synthetic or occurring naturally), which is capable of acting as a point of initiation of nucleic acid

synthesis or replication along a complementary strand when placed under conditions in which synthesis of a complementary stand is catalyzed by a polymerase.

The term "probe" refers to an oligonucleotide (synthetic or occurring naturally), that is significantly complementary to a "fragment" and forms a duplexed structure by hybridization with at least one strand of the fragment.

The term "complementary" is used to describe the relationship between nucleotide bases that are hybridizable to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength.

Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a  $T_m$  of 55°, can be used, e.g., 5X SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5X SSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher  $T_m$ , e.g., 40% formamide, with 5X or 6X SSC.

Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher  $T_m$ ) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., *supra*, 9.50-9.51, hereby incorporated by reference). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide

determines its specificity (see Sambrook et al., *supra*, 11.7-11.8, hereby incorporated by reference). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferably a minimum length for a hybridizable nucleic acid is at least about 15 contiguous nucleotides; more 5 preferably at least about 20 contiguous nucleotides; and most preferably the length is at least 30 contiguous nucleotides. Thus, where a "probe" or "primer" is "derived from" or corresponds to a "portion" of a nucleic acid fragment, the probe or primer or portion will preferably be at least about 15 contiguous nucleotides; more preferably at least about 20 contiguous nucleotides; and most preferably the 10 length is at least 30 contiguous nucleotides of the fragment from which it is derived. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

The term "amplification product" refers to portions of nucleic acid 15 fragments that are produced during a primer directed amplification reaction. Typical methods of primer directed amplification include polymerase chain reaction (PCR), ligase chain reaction (LCR) or Strand displacement Amplification (SDA). If PCR methodology is selected, the replication composition would include for example, nucleotide triphosphates, two primers with appropriate 20 sequences, DNA or RNA polymerase and proteins. These reagents and details describing procedures for their use in amplifying nucleic acids are provided in U.S. Patent No. 4,683,202 (1987, Mullis, et al.) and U.S. Patent No. 4,683,195 (1986, Mullis, et al.). If LCR methodology is selected, then the nucleic acid 25 replication compositions would comprise, for example, a thermostable ligase, e.g., *T. aquaticus* ligase, two sets of adjacent oligonucleotides wherein one member of each set is complementary to each of the target strands, Tris HCl buffer, KCl, EDTA, NAD, dithiothreitol and salmon sperm DNA. See, for example, Tabor et al., *Proc. Acad. Sci. U.S.A.*, 82, 1074-1078 (1985)).

The term "sequence analysis software" refers to any computer algorithm 30 or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software will include but is not limited to the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisc.), BLASTP, BLASTN, 35 BLASTX (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), and DNASTAR (DNASTAR, Inc., 1228 S. Park St. Madison, WI 53715 USA). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the

"default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

Standard recombinant DNA and molecular cloning techniques used here  
5 are well known in the art and are described by Sambrook, J., Fritsch, E. F. and  
Maniatis, T., Molecular Cloning: A Laboratory Manual, Second Edition, Cold  
Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) (hereinafter  
"Maniatis"); and by Silhavy, T. J., Bennan, M. L. and Enquist, L. W.,  
10 Experiments with Gene Fusions, Cold Spring Harbor Laboratory Cold Press  
Molecular Biology, published by Greene Publishing Assoc. and Wiley-  
Interscience (1987).

The present invention relates to unique 16S rDNA sequences which have  
been isolated from the bacteria very similar if not related to *Dehalococcoides*  
15 *ethenogenes*, which are associated with the ability of this bacteria to dechlorinate  
chlorinated organic compounds. The sequences were isolated from bacteria found  
in soil samples of various industrial sites that have been shown to contain bacteria  
that have the ability to dechlorinate chlorinated compounds. The sequences are  
useful for the identification new dechlorinating bacteria, as well as for sub-typing  
20 strains of *Dehalococcoides ethenogenes*.

Dechlorinating bacteria were isolated from the aquifer soil taken from  
around industrial sites by means well known in the art. Samples were maintained  
under anaerobic conditions and cultured in a suitable medium for the growth of  
anaerobic soil bacteria. Such culture procedures and media are common and well  
25 known in the art and are described in Manual of Methods for General  
Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W.  
Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American  
Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in  
30 Biotechnology: A Textbook of Industrial Microbiology, Second Edition, Sinauer  
Associates, Inc., Sunderland, MA (1989).

In order to enrich the cultured soil samples for dechlorinating bacteria, the  
samples were contacted with a low level of chlorinated organic compound. A  
number of chlorinated compounds are suitable for this purpose, including, but not  
limited to carbontetrachloride, tetrachloroethene, chloroform, dichloromethane,  
35 trichloroethene, dichloroethylene, vinyl chloride, and chloroaromatics, where  
chlorinated ethenes are preferred and TCE and PCE are preferred. Incubation  
proceeded for about six months, and cultures were analyzed periodically for the  
disappearance of the chlorinated organic and the appearance of degradation

products. Cultures demonstrating the ability to degrade chlorinated organics, were selected for further analysis.

Bacteria from dechlorinating cultures were removed by standard methods and total chromosomal DNA was isolated from the microorganisms through a bead mill homogenization procedure. A fragment of the 16S rRNA gene was amplified from the genomic DNA extract by PCR using 16S rDNA primers specific for dechlorinating microbes. The 16S rDNA PCR product was cloned and sequenced to confirm its identity (M. I. More et al. 1994. *Appl. Environ. Microbiol.*, 60, 1572-1580). Each raw 16S sequences obtained were assembled into a contig, and a consensus was manually constructed using Seqman II in DNASTar (DNASTar, Inc., Madison, WI). For each test sequence, a Pearson and Lipman similarity search was performed using the FASTA program in GCG (Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI). The nearest organism in similarity in 16S rRNA sequence to the test sequence was used as the nearest match for identification. Those 16S DNA gene sequences that were identified to be similar to the dechlorinating bacteria, *Dehalococcoides ethenogenes* DHE-195 (GenBank Accession No. AF004928), were aligned with selected 16s rRNA sequences extracted from the Ribosomal Database Project (Michigan State University) that were a representation of the major microorganism domains, Bacteria and Archeae in the Universal Phylogenetic Tree of Life. The sequences were aligned using MegAlign in DNASTar, using the default software parameters. From this alignment probable region for signature sequences were mapped. Then sequences from each region were tested against the Ribosomal Database (RDB) for unique sequences that could be signature sequences and utilized as PCR primes or detection probes.

Within the 16S rDNA profile defined by the comparison of the isolated dechlorinators, (see Figures 1 and 2) three signature regions showed considerable variation from the known sequences. Those regions were defined as extending from E1146 to E1156 (SEQ ID NO:1), from E180 to E227 (SEQ ID NO:8), and from E1001 to E1047 (SEQ ID NO: 30). All of the dechlorinating isolates of the present invention contained the sequence as set forth in SEQ ID NO:1, which is conspicuously absent from the sequence known in the art (Maymó-Gatell et al. (*Science*, 176:1568 (1997)).

Although a region similar to that defined by SEQ ID NO:8 is found in the literature sequence, there are significant variations at positions, E184, E190, E197, E200, E207, E216, and E221 as shown below in Formula I.

**Formula I.**

E184    E190    E198    E201    E208            E217    E222  
          |       |       |       |       |       |       |  
 5    TGTGRTGGGCY GACATAWGTY GGTCAYTAA AGCCGYAAGGYGC TTG    (SEQ ID NO:8)

With in the context the present invention Applicants have discovered that within the signature region defined by SEQ ID NO:8 and Formula I above, the R at position E184 may be A/G, the Y at position E190 may be C/T, the W at 10 position E198 may be A/T, and the Y's at position E201, E208, E217, and E222 may be T/C.

Similarly the region defined by SEQ ID NO:30 is also found in the literature but contains significant variations at positions, E1003, E1012, E1020, E1039, and E1040 as shown below in Formula II.

**15    Formula II.**

E1003            E1012    E1020                                  E1039  
          |           |       |    |  
 TGWAGTAGTGAACMGAAAGGGRAACGACCTGTTAACGTCAGGARMTTGCACA    (SEQ ID NO:30)  
 20    |  
     E1040

As with SEQ ID NO:8, Applicants have discovered that within the signature region defined by SEQ ID NO:30 and Formula II above, the W at 25 position E1003 may be A/T, at position E1012 the M may be A/C, at position E1020 the R may be A/G, at position E1039 the R may be A/G and , at position E1040 the M may be A/C.

Likewise, if the entire 16S rDNA profile is examined, it is seen that there are significant single base differences throughout the entire profile (Figures 1 and 30 2). These differences are illustrated in tabular form in Table 2. Accordingly a 16S rDNA profile sequence, having the following bases substitutions taken independently or together will be diagnostic for dechlorinating bacteria: E107=G, base E184=G, base E190=C, E 198=T, E201= T, E208=C, E217=T, E222=C, E264=C, E267=C, E291=T, E333= C, E420=C, E444=T, E631=A, E829=A, 35 E933=T, E934=T, E980=C, E1003=T, E1012=T, E1020=G, E1039=A, E1040=C, E1087=T, and E1114=C.

**Assay Methods**

The instant sequences may be used in a variety of formats for the detection of dechlorinating bacteria. The two most convenient formats will rely on methods

of nucleic acid hybridization or primer directed amplification methods such as PCR.

Nucleic Acid Hybridization Methods

The basic components of a nucleic acid hybridization test include a probe, 5 a sample suspected of containing a dechlorinating bacteria and a specific hybridization method. As noted above, probes of the present invention are single strand nucleic acid sequence which is complementary to the nucleic acid sequences to be detected. Probes are "hybridizable" to the nucleic acid sequence to be detected. The probe length can vary from 5 bases to tens of thousands of 10 bases, and will depend upon the specific test to be done. Only part of the probe molecule need be complementary to the nucleic acid sequence to be detected. In addition, the complementarity between the probe and the target sequence need not be perfect. Hybridization does occur between imperfectly complementary molecules with the result that a certain fraction of the bases in the hybridized 15 region are not paired with the proper complementary base. A probe may be composed of either RNA or DNA. The form of the nucleic acid probe may be a marked single strand molecule of just one polarity or marked single strand molecule having both polarities present. The form of the probe, like its length, will be determined by the type of hybridization test to be done.

20 The sample may or may not contain the organism of interest. The sample may take a variety of forms, including liquid such as water, or solid such as dust, or soil. The sample nucleic acid must be made available to contact the probe before any hybridization of probe and target molecule can occur. Thus the organism's RNA must be free from the cell and placed under the proper 25 conditions before hybridization can occur. Methods of in solution hybridization necessitate the purification of the RNA in order to be able to obtain hybridization of the sample rRNA with the probe. This has meant that to utilize the in solution method for detecting target sequences in a sample, the nucleic acids of the sample must first be purified to eliminate protein, lipids, and other cell components, and 30 then contacted with the probe under hybridization conditions. Method for the purification of the sample nucleic acid are common and well known in the art (Maniatis, *supra*).

35 Similarly, hybridization methods are well defined. Typically the probe and sample must be mixed under conditions which will permit nucleic acid hybridization. This involves contacting the probe and sample in the presence of an inorganic or organic salt under the proper concentration and temperature conditions. The probe and sample nucleic acids must be in contact for a long enough time that any possible hybridization between the probe and sample nucleic

acid may occur. The concentration of probe or target in the mixture will determine the time necessary for hybridization to occur. The higher the probe or target concentration the shorter the hybridization incubation time needed.

In one embodiment, hybridization assays may be conducted directly on bacterial lysates, without the need to extract the nucleic acids. This eliminates several steps from the sample-handling process and speeds up the assay. To perform such assays on crude cell lysates, a chaotropic agent is typically added to the cell lysates prepared as described above. The chaotropic agent stabilizes nucleic acids by inhibiting nuclease activity. Furthermore, the chaotropic agent allows sensitive and stringent hybridization of short oligonucleotide probes to RNA at room temperature [Van Ness and Chen (1991) *Nucl. Acids Res.* 19:5143-5151]. Suitable chaotropic agents include guanidinium chloride, guanidinium thiocyanate, sodium thiocyanate, lithium tetrachloroacetate, sodium perchlorate, rubidium tetrachloroacetate, potassium iodide, and cesium trifluoroacetate, among others. Typically, the chaotropic agent will be present at a final concentration of about 3M. If desired, one can add formamide to the hybridization mixture, typically 30-50% (v/v).

Alternatively, one can purify the rRNA prior to probe hybridization. A variety of methods are known to one of skill in the art (e.g., phenol-chloroform extraction, IsoQuick extraction (MicroProbe Corp., Bothell, WA), and others). Pre-hybridization purification is particularly useful for standard filter hybridization assays. Furthermore, purification facilitates measures to increase the assay sensitivity by incorporating *in vitro* RNA amplification methods such as self-sustained sequence replication (see for example Fahy et al. (1991) in PCR Methods and Applications, Cold Spring Harbor Laboratory Press, pp. 25-33) or reverse transcriptase PCR (Kawasaki (1990) in PCR Protocols: A Guide to Methods and Applications, M. A. Innis et al., eds., pp. 21-27). One can obtain amplified rRNA by using *in vitro* RNA amplification techniques as described in Fahy et al., *supra*; Kawasaki, *supra*. The exact procedure used is not crucial, provided that it does not amplify significant amounts of DNA, which would tend to obscure results.

Once the pre-rRNA is released from the cells, it can be detected by any of a variety of methods. The method of rRNA detection is not crucial to the invention. However, the most useful embodiments have at least some of characteristics of speed, convenience, sensitivity, and specificity. Direct DNA probe analysis is suitable, as is an *in vitro* RNA amplification method, such as 3SR, that employs labelled primers.

Various hybridization solutions can be employed. Typically, these comprise from about 20 to 60% volume, preferably 30%, of a polar organic solvent. A common hybridization solution employs about 30-50% v/v formamide, about 0.15 to 1M sodium chloride, about 0.05 to 0.1M buffers, such as sodium citrate, Tris-HCl, PIPES or HEPES (pH range about 6-9), about 0.05 to 0.2% detergent, such as sodium dodecylsulfate, or between 0.5-20 mM EDTA, FICOLL (Pharmacia Inc.) (about 300-500 kilodaltons), polyvinylpyrrolidone (about 250-500 kdal), and serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 mg/mL, fragmented nucleic DNA, e.g., calf thymus or salmon sperm DNA, or yeast RNA, and optionally from about 0.5 to 2% wt./vol. glycine. Other additives may also be included, such as volume exclusion agents which include a variety of polar water-soluble or swellable agents, such as polyethylene glycol, anionic polymers such as polyacrylate or polymethylacrylate, and anionic saccharidic polymers, such as dextran sulfate.

Nucleic acid hybridization is adaptable to a variety of assay formats. One of the most suitable is the sandwich assay format. The sandwich assay is particularly adaptable to hybridization under non-denaturing conditions. A primary component of a sandwich-type assay is a solid support. The solid support has adsorbed to it or covalently coupled to it immobilized nucleic acid probe that is unlabeled and complementary to one portion of the rRNA sequence. Preferred are those probes that hybridize to regions of the rRNA that have minimal secondary and tertiary interactions. The advantage of such probes is that the hybridization can be carried out without the additional step of heat denaturing the sample nucleic acid. For example, the hybridization can be carried out at room temperature.

The sandwich assay may be encompassed in an assay kit. This kit would include a first component for the collection of samples from soil such as vials for containment, and buffers for the disbursement and lysis of the sample. A second component would include media in either dry or liquid form for the hybridization of target and probe polynucleotides, as well as for the removal of undesirable and nonduplicated forms by washing. A third component includes a solid support (dipstick) upon which is fixed or to which is conjugated unlabeled nucleic acid probe(s) that is(are) complementary to a part of the precursor rRNA of the species of bacteria being tested. In the case of multiple target analysis more than one capture probe, each specific for its own rRNA, will be applied to different discrete regions of the dipstick. A fourth component would contain labeled probe that is complementary to a second and different region of the same rRNA strand to

which the immobilized, unlabeled nucleic acid probe of the third component is hybridized.

In another embodiment, the instant 16S rDNA sequence may be used as a 3' blocked detection probe in either a homogeneous or heterogeneous assay 5. format. For example a probe generated from the instant sequences may be 3' blocked or non-participatory and will not be extended by, or participate in, a nucleic acid amplification reaction. Additionally, the probe incorporates a label that can serve as a reactive ligand that acts as a point of attachment for the immobilization of the probe/analyte hybrid or as a reporter to produce detectable 10 signal. Accordingly, genomic or cDNA isolated from the test organism is amplified by standard primer-directed amplification protocols in the presence of an excess of the 16S rDNA 3' blocked detection probe to produce amplification products. Because the probe is 3' blocked, it does not participate or interfere with the amplification of the target. After the final amplification cycle, the detection 15 probe anneals to the relevant portion of the amplified DNA and the annealed complex is then captured on a support through the reactive ligand.

#### PCR Assay Methods

In an alternate embodiment the present sequences may be used as primers or to generate primers that may be used in primer directed nucleic acid 20 amplification to detect the presence of dechlorinating bacteria. A variety of primer directed nucleic acid amplification methods are known in the art including thermal cycling methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) as well as isothermal methods and strand displacement amplification (SDA). The preferred method is PCR. Typically, in PCR-type 25 amplification techniques, the primers have different sequences and are not complementary to each other. Depending on the desired test conditions, the sequences of the primers should be designed to provide for both efficient and faithful replication of the target nucleic acid. Methods of PCR primer design are common and well known in the art. (Thein and Wallace, "The use of 30 oligonucleotide as specific hybridization probes in the Diagnosis of Genetic Disorders", in *Human Genetic Diseases: A Practical Approach*, K. E. Davis Ed., (1986) pp. 33-50 IRL Press, Herndon, Virginia); Rychlik, W. (1993) In White, B. A. (ed.), Methods in Molecular Biology, Vol. 15, pages 31-39, PCR Protocols: Current Methods and Applications. Humania Press, Inc., Totowa, NJ.)

If a nucleic acid target is to be exponentially amplified, then two primers 35 are used each having regions complementary to only one of the stands in the target. After heat denaturation, the single-stranded target fragments bind to the respective primers which are present in excess. Both primers contain asymmetric

restriction enzyme recognition sequences located 5' to the target binding sequences. Each primer-target complex cycles through nicking and polymerization/displacement steps in the presence of a restriction enzyme, a DNA polymerase and the three dNTP's and one dNTP[aS] as discussed above. An in 5 depth discussion of SDA methodology is given by Walker et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89, 392, (1992).

Alternatively, asymmetric amplification can be used to generate the strand complementary to the detection probe. Asymmetric PCR conditions for producing single-stranded DNA would include similar conditions for PCR as 10 described however, the primer concentrations are changed with 50 pmol of the excess primer and 1 pmol of the limiting primer. It is contemplated that this procedure would increase the sensitivity of the method. This improvement in sensitivity would occur by increasing the number of available single strands for binding with the detection probe.

15 Within the context of the present invention primers will be designed to conserved regions of the 16S rDNA profile which are associated with dechlorination. The most significant of those regions are the sequences set forth in SEQ ID NO:1, SEQ ID NO:8 and SEQ ID NO:30.

Following amplification and prior to sequencing, the amplified nucleotide 20 sequence may be ligated to a suitable vector followed by transformation of a suitable host organism with said vector. One thereby ensures a more readily available supply of the amplified sequence. Alternatively, following amplification, the amplified sequence or a portion thereof may be chemically 25 synthesized for use as a nucleotide probe. In either situation the DNA sequence of the variable region is established using methods such as the dideoxy method (Sanger, F. et al. *Proc. Natl. Acad. Sci* (1977) 74, 5463-5467). The sequence obtained is used to guide the choice of the probe for the organism and the most appropriate sequence(s) is/are selected.

#### EXAMPLES

30 The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit 35 and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

GENERAL METHODS

Standard recombinant DNA and molecular techniques used in the Examples are well known in the art. Techniques suitable for use in the following examples may be found in Sambrook, J., Fritsch, E. F. and Maniatis, T.,

5. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) (hereinafter "Maniatis").

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General  
10 Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology, Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and  
15 materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

Manipulations of genetic sequences were accomplished using the suite of  
20 programs available from the Genetics Computer Group Inc. (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA), or the "on-line" Probe Match Program from the Ribosomal Database Project II (Michigan State University, East Lansing, MI). Where any sequence analysis software was used in  
25 the following examples, default values were used unless otherwise specified.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters.

EXAMPLE 1

- 30 Isolation And Characterization Of Dechlorinating Soil Organisms

Aquifer core samples were obtained by split spoon sampling at depths ranging from 10 to 80 ft, depending on the depth of the particular aquifer to be tested. The cores were taken in sterile stainless steel cylinders or placed in sterile glass vials. The core samples were immediately shipped to the laboratory at  
35 ambient temperatures and under anaerobic conditions. Upon arrival the samples were stored in an anaerobic glove bag (chamber) (Coy Laboratory Products Inc., Ann Arbor, MI), whose atmosphere was 10% H<sub>2</sub>, 5% CO<sub>2</sub> and 85% N<sub>2</sub>.

The laboratory microcosms were prepared in 250 mL Wheaton bottles (Wheaton Co., Millville, NJ) within the anaerobic chamber. Duplicate microcosms were prepared for the following conditions: Killed Control (live soil autoclaved for 1 hr on 2 consecutive days), Live soil, and Live soil + 0.05% yeast extract. Each microcosm contains 20% soil and 80% BTZ-3 media ( $\text{NH}_4\text{Cl}$ , 4.3 g/L;  $\text{KH}_2\text{PO}_4$ , 50 g/L;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 20 g/L;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 1 g/L; HEPES, 50 mM/L; mineral solution, 10 mL/L; resazurin 0.2%, 5 mL/L). The microcosm were filled to top such there was little or no headspace, and then stoppered with Teflon™ lined disks and crimp-sealed with aluminum seals (Wheaton Co., Millville, NJ). The resazurin addition permitted the visualization of low potential anaerobic conditions by a color change from pink to colorless. Each microcosm was spiked with 5 ppm from a PCE or TCE solution saturated in water. The microcosms were incubated on their sides in the anaerobic chamber, in the dark, at ambient room temperature (22°C) for up to 180 days.

Samples were analyzed the next day as time zero ( $t_0$ ) and then twice a week for the dechlorination of PCE or TCE and the formation of cisDCE, vinyl chloride or methane. All samples were taken in the anaerobic chamber by using a syringe mounted with a 23 gauge needle was use to puncture the Teflon™ septa to obtain a 5 mL liquid sample that was injected into a 10 mL headspace vial. Samples were tested using HP Headspace sampler 7694, HP5890 series II GC (FID detector, HP 5 capillary column #19091J-215), HP3365 Chemstation version A.03.34.

Figure 3 plots the concentration (parts per million; ppm) of chloroethenes in the microcosm medium as a function of time (days) and illustrates the dechlorination of chloroethenes. Dechlorination of PCE to TCE could be detected by GC/FID. Within two days with the formation of cisDCE from the dechlorination of TCE was detected. These results are found in the microcosms that has been amended with 0.05% yeast extract plus minimal salts media (BTZ-3 media). These results can also be seen in the microcosms that are amended with the minimal salts media alone. The difference is the dechlorination is slightly delayed. It takes four days before cisDCE is detected. Degradation of cisDCE would occur over the next two weeks. Vinyl chloride and ethene could only be detected at trace levels. The "Killed" control, did not show degradation of PCE or TCE during the duration of the experiment. Cell growth was shown by increase in the turbidity of the microcosm medium and by microscopic analysis.

EXAMPLE 2Generation of PCR Primers and Probes for the Amplification  
and Detection of the *Dehalococcoides ethenogenes* 16S rRNA Profiled

The detection and sequencing of the *Dehalococcoides ethenogenes*-like organisms used the set of PCR primers are shown in Table 1. The PCR primers were designed using signature sequence regions. To determine the location of these signature sequence, the *Dehalococcoides ethenogenes* sequence (GenBank No. AF004928)[SEQ ID NO:7] was aligned using MEGALIGN (DNAsstar, Madison, WI) or Pileup (Genetics Computer Group, Madison, WI) with 16S rRNA sequences from 100 organisms that represent most major domains, families and genera in the major kingdoms of Bacteria and Archaea. The conserved, variable, and highly variable regions could be delineated by boxing off the consensus sequences. Primer candidate sequences were manually picked from the variable and highly variable regions and then their uniqueness was determined by determining their potential as probes to a ribosomal sequence database sequences using the "on-line" Probe Match Program from the Ribosomal Database Project II (<http://www.cme.msu.edu/RDP/html/index.html>)RDPII, Michigan State University, East Lansing, MI). This analysis returned an overview of the matches between a probe and its potential target sequence, as a listing and as a phylogenetic overview. The program results showed the sequences that match the query sequence (if there are such sequences) and also showed sequences that had mismatches, deletions and insertions, citing the number and positions of the aberrations.

The sequences which were unique and passed this test as signature sequences were then designed as either a forward or reverse primer, usually dependent on their position in the sequence. The most unique sequence of the signature sequence (specificity) was designed into the 3' end in either type of primer. The selected primers are shown in Table 1.

The primers were synthesized using standard β-cyanoethyl phosphoramidite coupling chemistry on controlled pore glass (CPG) supports on automated DNA oligonucleotide synthesizer (Applied Biosystems Model 392, Perkin-Elmer, Foster City, CA)

The primers were tested after they were synthesized using PCR on samples taken from microcosms known to have *Dehalococcoides ethenogenes*-like organisms. The PCR products were sized on agarose electrophoresis and then cloned and sequenced to verify that the amplified sequences were *Dehalococcoides ethenogenes*-like 16S rRNA sequences.

TABLE 1  
Primers for *Dehalococcoides ethenogenes*

FP DHE 32	5'AAG TCGAACGGTCTTAAGCA3' SEQ ID NO:9
RP DHE422	5' CGTCATTATTCTTCCCTGTG 3' SEQ ID NO:10
FP DHE 958	5'GGGAAACGACCTGTTAAGTCA 3' SEQ ID NO:11
RP DHE 1212	5'GGATTAGCTCCAGTTCACACTG 3' SEQ ID NO:12
RP DHE 1076	5'AAATTAAC TAGCAACAAAGG 3' SEQ ID NO:13
FP DHE 795	5'GGAGTATCGACCCTCTTG 3' SEQ ID NO:14
FP DHE 774	5'GGGAGTATCGACCCTCTC 3' SEQ ID NO:15
FP DHE 946	5'AGTGAACCGAAAGGGAAA 3' SEQ ID NO:16
FP DHE 385	5'GGGTTGTAAACCTCTTTCAC 3' SEQ ID NO:17
RP DHE 806	5'GTTAGCTTCGGCACAGAGAG 3' SEQ ID NO:18
RP DHE 692	5'TCAGTGACAACCTAGAAAAC 3' SEQ ID NO:19
FP DHE1	5'GATGAACGCTAGCGGCG 3' SEQ ID NO:20
FP DHE 30	5'GTGCCTTATGCATGCAAG 3' SEQ ID NO:21
FP DHE 1187	5' AATAGGTTGCAACAGTGTGAA 3' SEQ ID NO:22
FP DHE 1175	5' AATGGACAGAACAAATAGGTTGC 3' SEQ ID NO:23
RP DHE 1381	5' GGCACATCGACTTCAAGTGTT 3'SEQ ID NO:24
RP DHE 1381	5' GGCACATCGACTTCAAGTGTT 3'SEQ ID NO:25
FP DHE 558	5' TAACCGGGACG(AT)GTCATTCA 3'SEQ ID NO:26
FP DHE 593	5' GAGTACAGCAGGAGAAAAC 3'SEQ ID NO:27
RP DHE 1394	5' CCTCCTTGC GGTTGGCACATC 3'SEQ ID NO:28
RP DHE 1090	5' GGCAGTCTCGCTAGAAAAT 3'SEQ ID NO:29

EXAMPLE 3

5      Using the *Dehalococcoides Ethenogenes*-Like Specific Primers  
to Detect These Organisms in Microcosms

Nucleic acids were extracted from the microcosm cultures by a bead mill homogenization procedure, FastDNA Spin Kit for Soil (Bio 101, Vista, CA), that was designed to isolate genomic DNA from all cell types. Approximately 10 mL of the microcosm culture was pelleted and resuspended in 500 ul of the culture media. The resuspended pellet was added to a 2.2 mL conical screw-cap tube containing 1.5 g of three differently sized glass and zirconia/silica beads (106 microns, 710-1180 microns). To the sample tubes, 978 ul of sodium phosphate buffer and 122 ul of MT buffer was added. The tubes were 10 homogenized for 30 seconds at speed 5.5 on a Fast Prep bead mill homogenizer. A clear supernatant was obtained by centrifuging the samples at 14,000 x g for 30 seconds. The supernatant was transferred to a clean microcentrifuge tube and 15

250 ul of PPS reagent was added and mixed. The resulting precipitate was pelleted through centrifugation at 14,000 x g for 5 minutes. The supernatant was transferred to a new microcentrifuge tube and 1 mL of binding matrix was added. The samples were placed on a rotator for 2 minutes and then sat on the benchtop 5 for 3 minutes to allow the settling of the silica matrix. Between 500-700 ul of the supernatant was removed and discarded. The remaining supernatant was used to resuspend the silica matrix and transferred to a spin filter. The spin filter was centrifuged for 1 minute at 14,000 x g and the flow-through decanted. The silica matrix was washed with 500 ul of SEWS-M buffer and centrifuged for 1 minute at 10 16,000 x g. The flow through was discarded and any residual buffer in the matrix was removed by a 2 minute centrifugation at 14,000 x g. The spin filter was placed in a catch tube and air dried for 5 minutes in a biological hood. The genomic DNA was eluted by adding 60 ul of sterile, deionized water, mixing the matrix and the water together with a pipet tip, and centrifuging for 1 minute at 15 14,000 x g.

The 16S rRNA gene for *Dehalococcoides ethenogenes* was detected by PCR amplification and gel electrophoresis. The 16S sequences were amplified using *Dehalococcoides ethenogenes* specific 16S rDNA primers shown in Table 1. All PCR amplifications were performed using the GeneAmp PCR kit with Taq 20 DNA polymerase (PE Applied Biosystems, Branchburg, NJ) in a Perkin Elmer 9600 thermal cycler. Amplification reactions contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 μM each deoxynucleoside triphosphate, 20 pmol each primer, 2.5 U of Taq polymerase, and 1 μL of the genomic extraction diluted 1:10 in a final reaction volume of 50 μL. The PCR 25 conditions were as follows: 2 minutes of denaturation at 95 °C, followed by 30 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C, 30 seconds at 72 °C. 8 μL of the PCR product was visualized on a 2% agarose gel (SeaKem GTG, FMC BioProducts, Rockland, ME) stained with ethidium bromide.

A direct detection protocol used 1 μL of the microcosm culture was 30 directly added to the PCR as described previously.

After the *Dehalococcoides ethenogenes*-like sequences were detected in the microcosm developed from contaminated soil, FP DHE 1 (SEQ ID NO:20), RP DHE 1330 (SEQ ID NO:12) were used to amplify a 1212 bp (or 1221 bp) fragment, which was cloned (using the PCR dA/T-Cloning System, Invitrogen, 35 Inc., CA) and sequenced (using Model 377 DNA Sequencer kit and system, Applied Biosystems, Perkin-Elmer, Foster City, CA). The sequence was assembled using the Seqman II program (DNAsstar, Inc., Madison, WI). The 16S rDNA sequence contig formed was compared to 16S rDNA sequences obtained

from microcosms developed from contaminated soils from other sites and the comparison is shown in Figure 4.

Figure 4 shows a gel of amplification products generated from PCR amplification of various *Dehalococcoides ethenogenes* isolated from a number of industrial sites contaminated with either PCE or TCE. All amplifications were carried out using primers SEQ ID NOs:17 paired with 19, and SEQ ID NOs:18 paired with 20. Lanes 1 and 12 carry the molecular weight markers. Lanes 2 and 3 are the PCR products generated from organisms isolated from soil containing PCE. Lanes 4, 5, 6, 7, 8 and 9 are the PCR products from organisms isolated from soil containing TCE. Lanes 10 and 11 contain negative PCR controls. As can be seen by the data all samples were detectable by the primers used.

The contiguous sequences from each site was unique, having 96 to 99% similarity to each other. The differences in the sequence are annotated in Table 2. A major difference exists in the consensus sequence that were obtained from all strains (CS) detected at contaminated sites and the reference sequence represented by the published sequence from strain DHE-195 strain (Table 2). At DHE (CS) positions 1088-1096 (*E. coli* coordinates E1146-E1156) there exists a nine base deletion. The sequence in CS strains reads ATTTTCTAGCGAGACTG (SEQ ID NO:31); in the DHE-195 strain it reads ATTTTCTAGCGAGACTG (SEQ ID NO:32) (the double underlined sequence is the sequence deleted in the CS strain sequences. Differences in sequence were found at six other base positions as shown below in Table 2.

TABLE 2

<i>E. coli</i>	DHE No.	DHE-195	DHE-PL	DHE-STF	DHE-DAB	DHE PIN	DHE DLL
28	1	1	1	1	1	1	1
107	66	A	66	A	66	C	66
184	144	A	144	A	144	G	144
190	150	T	150	C	150	C	150
198	157	A	157	A	157	T	157
201	160	C	160	T	160	T	160
208	167	T	167	C	167	C	167
217	176	C	176	T	176	T	176
222	181	T	181	T	181	C	181
264	226	T	226	C	226	T	226
267	*229	T	229	C	229	C	229
291+	254	d	253+	T	254	d	253+
333	*296	G	295	C	296	C	295
420	383	T	382	C	383	T	382
444	407	C	406	C	407	T	406

<i>E. coli</i>	DHE No.	DHE-195	DHE-PL	DHE-STF	DHE-DAB	DHE PIN	DHE DLL
542	*481	G 480	d 479+	d 480+	d 479+	d 479+	d 479+
631	571	T 570	T 569	T 570	A 569	A 569	T 569
829	769	G 768	G 767	A 768	G 767	G 767	G 767
933	874	G 873	T 872	G 873	G 872	G 872	G 872
934	875	C 874	T 873	C 874	C 873	C 873	C 873
980	*921	d 919+	C 919	C 920	C 919	C 919	C 919
1003	944	A 942	A 942	A 943	T 942	T 942	A 942
1012	955	C 953	C 953	C 954	T 953	T 953	C 953
1020	963	A 961	A 961	A 962	G 961	G 961	A 961
1039	984	G 982	G 982	G 983	A 982	A 982	G 982
1040	985	T 983	T 983	T 984	C 983	C 983	T 983
1087	*1033	G 103	T 1031	T 1032	T 1031	T 1031	T 1031
1114	*1060	d 1057+	C 1058	C 1059	C 1058	C 1058	C 1058
1144-56	1088-96	1086-94 ACTAGC	d 1085+	d 1086+	d 1085+	d 1085+	d 1085+

Legend: DHE No. = Consensus number; number (+) = last base coordinate before base a deletion; bold (red) bases are indicative of base sequences different from DHE 195; (\*), bold bases and blocks cells (base and coordinate) implies sequence of all 16S sequences isolates that are different from DHE 195

What is Claimed is:

1. An isolated 16S rDNA sequence indicative of a dechlorinating bacterial strain selected from the group consisting of:
  - (a) SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 SEQ ID NO:8 and SEQ ID NO:30;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS at 65°C; and
  - (c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

- 10 2. An isolated 16S rDNA sequence indicative of a dechlorinating bacterial strain selected from the group consisting of:
  - (a) SEQ ID NOS:9-29;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS at 65°C; and
  - 15 (c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

3. An isolated 16S rDNA sequence of Claim 1 wherein within the 16S DNA sequence the following bases, taken independently or together are:

base E107=G, base E184=G, base E190=C, E 198=T, E201= T, E208=C, 20 E217=T, E222=C, E264=C, E267=C, E291=T, E333= C, E420=C, E444=T, E631=A, E829=A, E933=T, E934=T, E980=C, E1003=T, E1012=T, E1020=G, E1039=A, E1040=C, E1087=T, and E1114=C.

4. An isolated 16S rDNA sequence indicative of a dechlorinating bacterial strain as set forth in SEQ ID NO:1.

- 25 5. An isolated bacterial strain comprising any one of the sequences of Claims 1, 3 or 4 wherein said strain has the ability to dechlorinate chlorinated compounds.

- 30 6. A method for identifying a dechlorinating bacterial strain comprising:

- (i) extracting genomic DNA from a cell suspected of being able to dechlorinate chlorinated compounds;
- (ii) probing the extracted genomic DNA with a probe derived from any one of the sequences of Claims 1, 2, 3 or 4 under suitable hybridization conditions;

wherein the identification of a hybridizable nucleic acid fragment confirms the presence 35 of a bacteria capable of dechlorinating chlorinated compounds.

7. A method for identifying a dechlorinating bacterial strain comprising:

- (i) extracting genomic DNA from a cell suspected of being able to dechlorinate chlorinated compounds; and

- (ii) amplifying the extracted genomic DNA with at least one oligonucleotide primer corresponding to a portion of any one of the sequences of Claims 1, 2, 3 or 4 such that amplification products are generated;
- 5 wherein the presence of amplification products confirms the presence of a bacteria capable of dechlorinating chlorinated compounds.
8. A method for the dechlorination of chlorinated compounds comprising contacting a chlorinated compound with the isolated bacterial strain of Claim 5 under conditions for the dechlorination to occur.
- 10 9. A method according to Claim 8 wherein said dechlorinating compound is selected from the group consisting of carbontetrachloride, tetrachloroethene, chloroform, dichloromethane, trichloroethene, dichloroethylene, vinyl chloride, and chloroaromatics.

## FIG. 1-1

DHE seq alignment1.msf MSF: 1223

Name : Deh_ _eth. (cornell)								
Name : Deh_ _eth. (stf)								
Name : Dehal_ _eth. (pl) . _seq								
Name : Dehlo_ _eth. (d11) .. _seq								
Name : Dehlo_ _eth. (dab) .								
Name : Dehlo_ _eth. (pin) . _seq								
//								
Deh_ _eth. (cornell)	1	GATGAAACGGCTAGGGCGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
Deh_ _eth. (stf)		GATGAAACGGCTAGGGGGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
Dehal_ _eth. (pl) . _seq	60	GATGAAACGCTAGGGGGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
Dehlo_ _eth. (d11) .. _seq		GATGAAACGCTAGGGGGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
Dehlo_ _eth. (dab) . _seq		GATGAAACGCTAGGGGGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
Dehlo_ _eth. (pin) . _seq		GATGAAACGCTAGGGGGTGCCTTATGCATGCAAGTCGAACGGTCTTAAGCAATTAAAGATA						
//								
Deh_ _eth. (cornell)	61	GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
Deh_ _eth. (stf)		GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
Dehal_ _eth. (pl) . _seq		GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
Dehlo_ _eth. (d11) .. _seq	120	GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
Dehlo_ _eth. (dab) . _seq		GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
Dehlo_ _eth. (pin) . _seq		GTGGCAAACGGGTGAGTAACGGTAAGTAACCTACCTCTAAGTGGGATAGCTTCGGGA						
//								
Deh_ _eth. (cornell)	121	AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						
Deh_ _eth. (stf)		AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						
Dehal_ _eth. (pl) . _seq		AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						
Dehlo_ _eth. (d11) .. _seq		AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						
Dehlo_ _eth. (dab) . _seq	180	AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						
Dehlo_ _eth. (pin) . _seq		AACTGAAGGTAAATACCGCATGTTGATGGCTGACATAAGTCGGTCATTAAGCCGAAGG						

2/17

FIG. 1-2

181	Deh_eth_._(cornell)	TTGCTTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAATGGTCTACCAAGGCT
	Deh_eth_._(stf)	TGCTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAACGGCTACCAAGGCT
	Dehal_.eth_.(p1) . _seq	TGCTTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAATGGCCTACCAAGGCT
	Dehal_.eth_.(d11) . _seq	TGCTTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAACGGCCTACCAAGGCT
	Dehlo_.eth_.(dab) . _seq	CGCTTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAATGGCCTACCAAGGCT
	Dehlo_.eth_.(pin) . _seq	CGCTTGGTGAGGGCCTTGGTCCGATTAGCTAGTGGTGGGTAATGGCCTACCAAGGCT
240		
241	Deh_eth_._(cornell)	TCGATCGGTAGCT . GGCTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
	Deh_eth_._(stf)	TCGATCGGTAGCTTGGTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
	Dehal_.eth_.(p1) . _seq	TCGATCGGTAGCT . GGCTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
	Dehal_.eth_.(d11) . _seq	TCGATCGGTAGCT . GGCTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
	Dehlo_.eth_.(dab) . _seq	TCGATCGGTAGCT . GGCTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
	Dehlo_.eth_.(pin) . _seq	TCGATCGGTAGCT . GGCTCTGAGAGGATGATCAGCCACACTGGACTGAGACACGGGCCAG
300		
301	Deh_eth_._(cornell)	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
	Deh_eth_._(stf)	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
	Dehal_.eth_.(p1) . _seq	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
	Dehal_.eth_.(d11) . _seq	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
	Dehlo_.eth_.(dab) . _seq	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
	Dehlo_.eth_.(pin) . _seq	ACTCCTACGGGAGGCAGCAAGGAATCTGGCCAATGGCGAAAGCCTGACCCAGCAA
360		
361	Deh_eth_._(cornell)	CGCCCGGTAGGGATGAAGGGCTTTCAGGGTTGTAACACCTCTTTCAGGGAAAGAATAATG
	Deh_eth_._(stf)	CGCCCGGTAGGGATGAAGGGCTCTGGGGTCTGGGGTAAACCTCTTTCAGGGAAAGAATAATG
	Dehal_.eth_.(p1) . _seq	CGCCCGGTAGGGATGAAGGGCTCTGGGGTAAACCTCTTTCAGGGAAAGAATAATG
	Dehal_.eth_.(d11) . _seq	CGCCCGGTAGGGCTGAAGGGCTCTGGGGTAAACCTCTTTCAGGGAAAGAATAATG
	Dehlo_.eth_.(dab) . _seq	CGCCCGGTAGGGCTGAAGGGCTCTGGGGTAAACCTCTTTCAGGGAAAGAATAATG
	Dehlo_.eth_.(pin) . _seq	CGCCCGGTAGGGCTGAAGGGCTCTGGGGTAAACCTCTTTCAGGGAAAGAATAATG
420		

3/17

## FIG. 1-3

421                          Deh\_eth\_.(cornell)        ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG  
                         Deh\_eth\_.(stf)            ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG  
                         Dehal\_.eth\_.(pl) . . . seq    ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG  
                         Dehal\_.eth\_.(d11) . . . seq    ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG  
                         Dehlo\_.eth\_.(dab) . . . seq    ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG  
                         Dehlo\_.eth\_.(pin) . . . seq    ACGGTACCTGTGGATAAGCTTCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGG

481                          Deh\_eth\_.(cornell)        GAAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG  
                         Deh\_eth\_.(stf)            .AAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG  
                         Dehal\_.eth\_.(pl) . . . seq    .AAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG  
                         Dehal\_.eth\_.(d11) . . . seq    .AAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG  
                         Dehlo\_.eth\_.(dab) . . . seq    .AAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG  
                         Dehlo\_.eth\_.(pin) . . . seq    .AAGCAAGCGTTATCCGGATTATTGGCGTAAAGTGACGGTAGGTGGCTTTCAAGTTG

541                          Deh\_eth\_.(cornell)        GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA  
                         Deh\_eth\_.(stf)            GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA  
                         Dehal\_.eth\_.(pl) . . . seq    GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA  
                         Dehal\_.eth\_.(d11) . . . seq    GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA  
                         Dehlo\_.eth\_.(dab) . . . seq    GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA  
                         Dehlo\_.eth\_.(pin) . . . seq    GATGTGAAATTCCGGCTTAACCGGGACGTGTCAATTCAATACTGTGGACTAGAGTACA

601                          Deh\_eth\_.(cornell)        GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC  
                         Deh\_eth\_.(stf)            GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC  
                         Dehal\_.eth\_.(pl) . . . seq    GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC  
                         Dehal\_.eth\_.(d11) . . . seq    GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC  
                         Dehlo\_.eth\_.(dab) . . . seq    GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC  
                         Dehlo\_.eth\_.(pin) . . . seq    GCAGGAGAAAACGGAATTCCGGTGTAGTGGTAAATGGCTAGATATGGGAGGAACACC

4/17

FIG. 1-4

661	Deh_eth_(cornell)	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	720
	Deh_eth_(stf)	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	
	Dehal_eth_(p1) . seq	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	
	Dehal_eth_(dll) . seq	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	
	Dehlo_eth_(dab) . seq	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	
	Dehlo_eth_(pin) . seq	AGAGGGCGAAGGGGGTTTCTTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGGGGAGCC	
721	Deh_eth_(cornell)	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	780
	Deh_eth_(stf)	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	
	Dehal_eth_(p1) . seq	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	
	Dehalo_eth_(dll) . seq	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	
	Dehlo_eth_(dab) . seq	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	
	Dehlo_eth_(pin) . seq	AACAGAAATTAGATACTCTGGTAAGTCCACGCCCTTAACATGGACACTAGGTATAGGGAGT	
781	Deh_eth_(cornell)	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	840
	Deh_eth_(stf)	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	
	Dehal_eth_(p1) . seq	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	
	Dehalo_eth_(dll) . seq	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	
	Dehlo_eth_(dab) . seq	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	
	Dehlo_eth_(pin) . seq	ATCGACCCCTCTCTGTGCCGAAGCTAACGCCTTAAGTGTCCCCGCCTGGGAGTACGGTTCGC	
841	Deh_eth_(cornell)	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCGACAAGCAGGGAGCGCTGTGGTTAA	900
	Deh_eth_(stf)	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCGACAAGCAGGGAGCGCTGTGGTTAA	
	Dehal_eth_(p1) . seq	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCCTAACAGCAGGGAGCGCTGTGGTTAA	
	Dehalo_eth_(dll) . seq	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCGACAAGCAGGGAGCGCTGTGGTTAA	
	Dehlo_eth_(dab) . seq	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCGACAAGCAGGGAGCGCTGTGGTTAA	
	Dehlo_eth_(pin) . seq	AAGGCTAAAAGTCAAAGGAATTGACGGGGCCGACAAGCAGGGAGCGCTGTGGTTAA	

## FIG. 1-5

Deh_eth_(cornell) Deh_eth_(stf) Dehal_eth_(pl) . _seq Dehalo_eth_(dll) .. seq Dehlo_ _eth_(dab) . seq Dehlo_ _eth_(pin) . seq	901 TTCGATGCTACAGGAAGAAC . TTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAACCTTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAAGAACCTTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAACCTTACCAAGATTGACATGCATGTAGTAGTGTGAACTGAAG TTCGATGCTACAGGAACCTTACCAAGATTGACATGCATGTAGTAGTGTGAACTGAAG	960 TTCGATGCTACAGGAAGAAC . TTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAAGAACCTTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAAGAACCTTACCAAGATTGACATGCATGAAGTAGTGTGAACCAGAAAG TTCGATGCTACAGGAAGAACCTTACCAAGATTGACATGCATGTAGTAGTGTGAACTGAAG TTCGATGCTACAGGAAGAACCTTACCAAGATTGACATGCATGTAGTAGTGTGAACTGAAG
Deh_eth_(cornell) Deh_eth_(stf) Dehalo_eth_(pl) . _seq Dehalo_eth_(dll) .. seq Dehlo_ _eth_(dab) . seq Dehlo_ _eth_(pin) . seq	961 GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG	1020 GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG GGAAACGCCCTGTTAAGTCAGGAGTTGCACAGGTGGCTGCATGGCTCGTCAAGCTCGTG
Deh_eth_(cornell) Deh_eth_(stf) Dehalo_eth_(pl) . _seq Dehalo_eth_(dll) .. seq Dehlo_ _eth_(dab) . seq Dehlo_ _eth_(pin) . seq	1021 CCGTGAGGGTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC	1080 CCGTGAGGGTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC CCGTGAGGGTGGTTGGTTAAGTCTGCACAGGAGGCCAAC . TTGTTGCTAGTTAAATTTC
Deh_eth_(cornell) Deh_eth_(stf) Dehalo_eth_(pl) . _seq Dehalo_eth_(dll) .. seq Dehlo_ _eth_(dab) . seq Dehlo_ _eth_(pin) . seq	1081 TAGCGAGACTAGCGAGAACCTGGGGAGGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC	1140 TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC TAGCGAG . . . . . ACTGCCCGCGAACCGGGAGGAAGGGGGATGACGGTCAAAGTC

6/17

## FIG. 1-6

1141	Deh_ _eth. (cornell) AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA Deh_ _eth. (stf) AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA Dehal_ _eth. (pl) . . seq AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA Dehlo_ _eth. (d11) . . seq AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA Dehlo_ _eth. (dab) . . seq AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA Dehlo_ _eth. (pin) . . seq AGCATGGCCTTTATATCTTGGGCTACACACGGCTACAATGGACAGAACAAATAGGTTGCA	1200
1201	Deh_ _eth. (cornell) ACAGTGTGAACCTGGAGCTAATCC Deh_ _eth. (stf) ACAGTGTGAACCTGGAGCTAATCC Dehal_ _eth. (pl) . . seq ACAGTGTGAACCTGGAGCTAATCC Dehlo_ _eth. (d11) . . seq ACAGTGTGAACCTGGAGCTAATCC Dehlo_ _eth. (dab) . . seq ACAGTGTGAACCTGGAGCTAATCC Dehlo_ _eth. (pin) . . seq ACAGTGTGAACCTGGAGCTAATCC	1223
		Seq. I.D. No 7 Seq. I.D. No. 3 Seq. I.D. No. 2 Seq. I.D. No. 6 Seq. I.D. No. 4 Seq. I.D. No. 5

7/17

FIG. 2-1

112/10/98 BECAlignmentG.msf	MSF:	1576	
Name: E.coli.rRNA			
Name: Deh_eth_(cornell)			
Name: Deh_eth_(stf)			
Name: Dehal_ _eth_(pl) _ seq			
Name: Dehal_ _eth_(dab) _ seq			
Name: Dehlo_ _eth_(pin) _ seq			
Name: Dehlo_ _eth_(dll) _ seq			
/ /			
			60
E. coli.rRNA	AATTGAAGAGTTGATCATGGCTCAGATTGAACGCCTGGGGCAGGCCCTAACACATGCCA		
Deh_eth_(cornell)	.....		
Deh_eth_(stf)	.....		
Dehal_ _eth_(pl) _ seq	.....		
Dehal_ _eth_(dab) _ seq	.....		
Dehlo_ _eth_(pin) _ seq	.....		
Dehlo_ _eth_(dll) _ seq	.....		
			61
E. coli.rRNA	AAATTGAAGAGTTGATCATGGCTCAGATTGAACGCCTGGGGCAGGCCCTAACACATGCCA		
Deh_eth_(cornell)	.....		
Deh_eth_(stf)	.....		
Dehal_ _eth_(pl) _ seq	.....		
Dehal_ _eth_(dab) _ seq	.....		
Dehlo_ _eth_(pin) _ seq	.....		
Dehlo_ _eth_(dll) _ seq	.....		
			62
E. coli.rRNA	GTCGAACCGTAACAGGAAGCTTGCTTCTTGTGACGAGTGGCGGACGGGTGAGTAA		
Deh_eth_(cornell)	GTCGAACCGTCTTAAGCAA...TTAA.....		
Deh_eth_(stf)	GTCGAACCGTCTTAAGCAA...TTAA.....		
Dehal_ _eth_(pl) _ seq	GTCGAACCGTCTTAAGCAA...TTAA.....		
Dehal_ _eth_(dab) _ seq	GTCGAACCGTCTTAAGCAA...TTAA.....		
Dehlo_ _eth_(pin) _ seq	GTCGAACCGTCTTAAGCAA...TTAA.....		
Dehlo_ _eth_(dll) _ seq	GTCGAACCGTCTTAAGCAA...TTAA.....		

8/17

FIG. 2-2

236

180	E. coli. rrnAB	TAACGTCCAAAGACCAAAAGAGGGGACCTTCGGGCCCTTGCATCGGATGTG...CCCA
	Deh_ethyl.(cornell)	TGTGATGGCTGAC.ATAAGTGGTTCATTAAGCCGAAAGGTGCTTGTAGGGCTTG
	Deh_ethyl.(stf)	TGTGGTGGCCGAC.ATAAGTGGTTCAACTAAAGCCGTAAGGTGCTTGTAGGGCTTG
	Dehalo_ethyl.(pl)	seq
	Dehalo_ethyl.(dab)	seq
	Dehalo_ethyl.(pin)	seq
	Dehalo_ethyl.(dll)	seq

E. coli . rRNA	GATGGGATTAGCTAGGTGGGTAACGGCTCACCTAGGGACGATCCCTAGCT . GGTC
Deh_ eth . (cornell)	CGTCCGATTAGCTAGGTGGGGTAATGGCTACCAAGGCTCGATGGTAGCT . GGTC
Deh_ eth . (stf)	CGTCCGATTAGCTAGGTGGGGTAACGGCCTACCAAGGCTCGATGGTAGCTGGTC
Dehal . eth . (p1)	. seq
Dehalo . eth . (dab)	. seq
Dehalo . eth . (pin)	. seq
Dehalo . eth . (dl1)	. seq

9/17

## FIG. 2-3

296	<b>E.coli.rRNA B</b> Deh_eth_(cornell) TGAGAGGGATGACCAAGCCACACTGGAAACTGAGAACACGGTCCAGACTCCTACGGGGAGC Deh_eth_(stf) TGAGAGGGATGATCAGCCACACTGGGACTGAGAACACGGGCCAGACTCCTACGGGGAGC Dehal_ _eth_ _ (pl) . _ seq Dehalo_ _eth_ _ (dab) . _ seq Dehalo_ _eth_ _ (eth) . _ seq Dehalo_ _eth_ _ (pin) . _ seq Dehalo_ _eth_ _ (dll) . _ seq	355	TGAGAGGGATGATCAGCCACACTGGGACTGAGAACACGGGCCAGACTCCTACGGGGAGC TGAGAGGGATGATCAGCCACACTGGGACTGAGAACACGGGCCAGACTCCTACGGGGAGC TGAGAGGGATGATCAGCCACACTGGGACTGAGAACACGGGCCAGACTCCTACGGGGAGC TGAGAGGGATGATCAGCCACACTGGGACTGAGAACACGGGCCAGACTCCTACGGGGAGC
356	<b>E.coli.rRNA B</b> Deh_eth_(cornell) AGTGGGGAAATTGCACAATGGGCAAGCCTGATGCAGCCATGCCCGTGTATGAAGAA Deh_eth_(stf) AGCAAGGAATCTGGCAATTGGGCAATGGGCAAAGCTGACCCAGAACGCCGGTGAAGGAA Dehal_ _eth_ _ (pl) . _ seq Dehalo_ _eth_ _ (dab) . _ seq Dehalo_ _eth_ _ (eth) . _ seq Dehalo_ _eth_ _ (pin) . _ seq Dehalo_ _eth_ _ (dll) . _ seq	415	AGTGGGGAAATTGCACAATGGGCAAGCCTGATGCAGCCATGCCCGTGTATGAAGAA AGCAAGGAATCTGGCAATTGGGCAATGGGCAAAGCTGACCCAGAACGCCGGTGAAGGAA AGCAAGGAATCTGGCAATTGGGCAATGGGCAAAGCTGACCCAGAACGCCGGTGAAGGAA AGCAAGGAATCTGGCAATTGGGCAATGGGCAAAGCTGACCCAGAACGCCGGTGAAGGAA AGCAAGGAATCTGGCAATTGGGCAATGGGCAAAGCTGACCCAGAACGCCGGTGAAGGAA
416	<b>E.coli.rRNA B</b> Deh_eth_(cornell) GGCTTCGGGTTAAAGTACTTTCAAGGGGAGGAAGGTAATAACCTTTGC Deh_eth_(stf) GGCTCTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . . Dehal_ _eth_ _ (pl) . _ seq Dehalo_ _eth_ _ (dab) . _ seq Dehalo_ _eth_ _ (eth) . _ seq Dehalo_ _eth_ _ (pin) . _ seq Dehalo_ _eth_ _ (dll) . _ seq	475	GGCTTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . . GGCTCTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . . GGCTCTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . . GGCTCTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . . GGCTCTCGGGTTGTAAACCTCTTTCAAGGGAGAA. . . . . TAAT. . . . .

10/17

## FIG. 2-4

476      E. coli. rrnaB      TCATTGACGTTACCCGGAGAAGAACCGGCTAACTCCGTGCCAGCAGCCGGTAATA  
 Deh\_ eth\_. (cornell)      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA  
 Deh\_ eth\_. (stf)      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA  
 Dehal\_ .eth\_. (pl) . seq      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA  
 Dehalo\_ .eth\_. (dab) . seq      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA  
 Dehlo\_ .eth\_. (pin) . seq      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA  
 Dehlo\_ .eth\_. (dl1) . seq      .... GACGGTACCTGTGGAATAAGCTTCGGCTAAACTACGTGCCAGCAGCCGGTAATA

535      E. coli. rrnaB      CCGAGGGT. GCAAGCGTTAATCGGAATTACTGGCGTAAAGGCCACGCAGGCCGGTTTGT  
 Deh\_ eth\_. (cornell)      CGTAGGGAGCCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Deh\_ eth\_. (stf)      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehal\_ .eth\_. (pl) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehalo\_ .eth\_. (dab) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehlo\_ .eth\_. (pin) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehlo\_ .eth\_. (dl1) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC

594      E. coli. rrnaB      CCGAGGGT. GCAAGCGTTAATCGGAATTACTGGCGTAAAGGCCACGCAGGCCGGTTTGT  
 Deh\_ eth\_. (cornell)      CGTAGGGAGCCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Deh\_ eth\_. (stf)      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehal\_ .eth\_. (pl) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehalo\_ .eth\_. (dab) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehlo\_ .eth\_. (pin) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC  
 Dehlo\_ .eth\_. (dl1) . seq      CGTAGG. AAGCAAGCGTTATCCGGATTATGGCGATTAAAGTGAAGCTAGGTGGCTCTTC

595      E. coli. rrnaB      AAGTCAGATGTGAAATCCCGGGCTCAACCTGGAACTGCACTGTGATACTGGCAAGCTTG  
 Deh\_ eth\_. (cornell)      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGTTGTCATTCAATACTGTGGACTAG  
 Deh\_ eth\_. (stf)      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGTTGTCATTCAATACTGTGGACTAG  
 Dehal\_ .eth\_. (pl) . seq      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGTTGTCATTCAATACTGTGGACTAG  
 Dehalo\_ .eth\_. (dab) . seq      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGAGTCATTCAATACTGTGGACTAG  
 Dehlo\_ .eth\_. (pin) . seq      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGAGTCATTCAATACTGTGGACTAG  
 Dehlo\_ .eth\_. (dl1) . seq      AAGTGGATGTGAAATTCCCGGGCTTAACCGGACGAGTCATTCAATACTGTGGACTAG

11/17

FIG. 2-5

655	E. coli .rrnaB Deh_eth_(cornell) Deh_eth_(stf)	AGTCTCGTAGGGGGTAGAATTCCAGGTGAAATGCGTAGAGATCTGGAGG AGTCAGCAGGAGAAAACGGAATTCCCGGTAGTGGTAAATGGTAGATATCGGGAGG
715	E. coli .rrnaB Deh_eth_(cornell) Deh_eth_(stf)	AATAACCGGGGGGAAGGGCCCCCTGGACGAAGACTGACGCTCAGGTGGCAAAGCGTGG AACACCAGAGGGGAAGGGTTTCTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGG
774	E. coli .rrnaB Deh_eth_(pl) Dehlo._eth.(dab) Dehlo._eth.(pin) Dehlo._eth.(d11)	AAACACCAAGAGGGGAAGGGGTTTCTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGG AACACCAAGAGGGGAAGGGGTTTCTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGG AACACCAAGAGGGGAAGGGGTTTCTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGG AACACCAAGAGGGGAAGGGGTTTCTAGGTTGTCACTGACACTGAGGCTCGAAAGCGTGG
755	E. coli .rrnaB Deh_eth_(cornell) Deh_eth_(stf)	GGAGGAAACAGGATTAGATACTCCCTGGTAGTCCACGCCGTAACCGATGTCGACTGGAGGT GGAGCGAACAGAAATTAGATACTCTGGTAGTCCACGCCCTTAACACTATGGACACTAGGTATA
834	E. coli .rrnaB Dehlo._eth.(pl) Dehlo._eth.(dab) Dehlo._eth.(pin) Dehlo._eth.(d11)	GGAGCGAACAGAAATTAGATACTCTGGTAGTCCACGCCCTTAACACTATGGACACTAGGTATA GGAGCGAACAGAAATTAGATACTCTGGTAGTCCACGCCCTTAACACTATGGACACTAGGTATA GGAGCGAACAGAAATTAGATACTCTGGTAGTCCACGCCCTTAACACTATGGACACTAGGTATA GGAGCGAACAGAAATTAGATACTCTGGTAGTCCACGCCCTTAACACTATGGACACTAGGTATA

12/17

## FIG. 2-6

835	E.coli.rnab	TGTGCCCTTGGGTTGGCTT	CCGGAGCTAACGGGTTAACGTCGACCGCCCTGGGAGTAC
Deh_	eth. (cornell)	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
Deh_	eth. (stf)	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
Dehal_	eth. (pl) . seq	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
Dehalo_	eth. (dab) . seq	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
Dehalo_	eth. (pin) . seq	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
Dehalo_	eth. (dll) . seq	GGGAGTATCGACCCTCTCTGTGCCGAAGCTAACGGCTTAAAGTGTCCCCTGGGAGTAC	
893			
901	E.coli.rnab	GGCCGCAAGGTTAAACTCAAATGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Deh_	eth. (cornell)	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Deh_	eth. (stf)	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Dehal_	eth. (pl) . seq	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Dehalo_	eth. (dab) . seq	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Dehalo_	eth. (pin) . seq	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
Dehalo_	eth. (dll) . seq	GTCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCCCACAGGGGGTGGAGCATGTC	
953			
954	E.coli.rnab	GTTAATTGATGCAACCGGAAGAACCTTACCTGGCTTGACATCCACCGA..AGTTTTC	
Deh_	eth. (cornell)	GTTAATTGATGCTACAGAAGAAC..TTACCAAGATTGACATGCATGAAGTAGTGTGAAC	
Deh_	eth. (stf)	GTTAATTGATGCTACAGAAGAACCTTACAGAAGAACCTAACAAAGATTTGACATGCATGAAGTAGTGTGAAC	
Dehal_	eth. (pl) . seq	GTTAATTGATGCTACAGAAGAACCTAACAAAGATTTGACATGCATGAAGTAGTGTGAAC	
Dehalo_	eth. (dab) . seq	GTTAATTGATGCTACAGAAGAACCTAACAAAGATTTGACATGCATGAAGTAGTGTGAAC	
Dehalo_	eth. (pin) . seq	GTTAATTGATGCTACAGAAGAACCTAACAAAGATTTGACATGCATGAAGTAGTGTGAAC	
Dehalo_	eth. (dll) . seq	GTTAATTGATGCTACAGAAGAACCTAACAAAGATTTGACATGCATGAAGTAGTGTGAAC	
1011			

13/17

## FIG. 2-7

1012	<b>E.coli.rRNA B</b> Deh_ _eth_ _ (cornell) CGAAAGGGAAACGACCTGTGTTAAGTCAGGAGTTGGCACAGGGCATGGCTCGTCAG Deh_ _eth_ _ (stf) CGAAAGGGAAACGACCTGTGTTAAGTCAGGAGTTGGCACAGGGCATGGCTCGTCAG Dehal_ _eth_ _ (pl) . _seq CGAAAGGGAAACGACCTGTGTTAAGTCAGGAGTTGGCACAGGGCATGGCTCGTCAG Dehal_ _eth_ _ (dab) . _seq TGAAAGGGAAACGACCTGTGTTAAGTCAGGAAACTTGCACAGGTGCTGCAATGGCTGTGCTCAG Dehal_ _eth_ _ (pin) . _seq TGAAAGGGAAACGACCTGTGTTAAGTCAGGAAACTTGCACAGGTGCTGCAATGGCTGTGCTCAG Dehal_ _eth_ _ (dl1) . _seq CGAAAGGGAAACGACCTGTGTTAAGTCAGGAGTTGGCACAGGTGCTGCAATGGCTGTGCTCAG
1068	
1069	<b>E.coli.rRNA B</b> Deh_ _eth_ _ (cornell) CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCG Deh_ _eth_ _ (stf) CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCG Dehal_ _eth_ _ (pl) . _seq CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCGTAGTTA. Dehal_ _eth_ _ (dab) . _seq CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCGTAGTTA. Dehal_ _eth_ _ (pin) . _seq CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCGTAGTTA. Dehal_ _eth_ _ (dl1) . _seq CTCGTGCCGTGAGGTGGTTAAGTCCTGCAACGAGCCAACCCATTATCCTTGTGCGTAGTTA
1128	
1129	<b>E.coli.rRNA B</b> Deh_ _eth_ _ (cornell) AATTTTCTAGC. GAG. ACT. .AGCGAGACTGCC. CGCGAAACGGGGAGGAAGGTGGGG Deh_ _eth_ _ (stf) .AATTTTCTAGC. GAG. . . . .ACTGCC. CGCGAAACGGGGAGGAAGGTGGGG Dehal_ _eth_ _ (pl) . _seq .AATTTTCTAGC. GAG. . . . .ACTGCC. CGCGAAACGGGGAGGAAGGTGGGG Dehal_ _eth_ _ (dab) . _seq .AATTTTCTAGC. GAG. . . . .ACTGCC. CGCGAAACGGGGAGGAAGGTGGGG Dehal_ _eth_ _ (pin) . _seq .AATTTTCTAGC. GAG. . . . .ACTGCC. CGCGAAACGGGGAGGAAGGTGGGG Dehal_ _eth_ _ (dl1) . _seq .AATTTTCTAGC. GAG. . . . .ACTGCC. CGCGAAACGGGGAGGAAGGTGGGG
1187	

14/17

FIG. 2-8

15/17

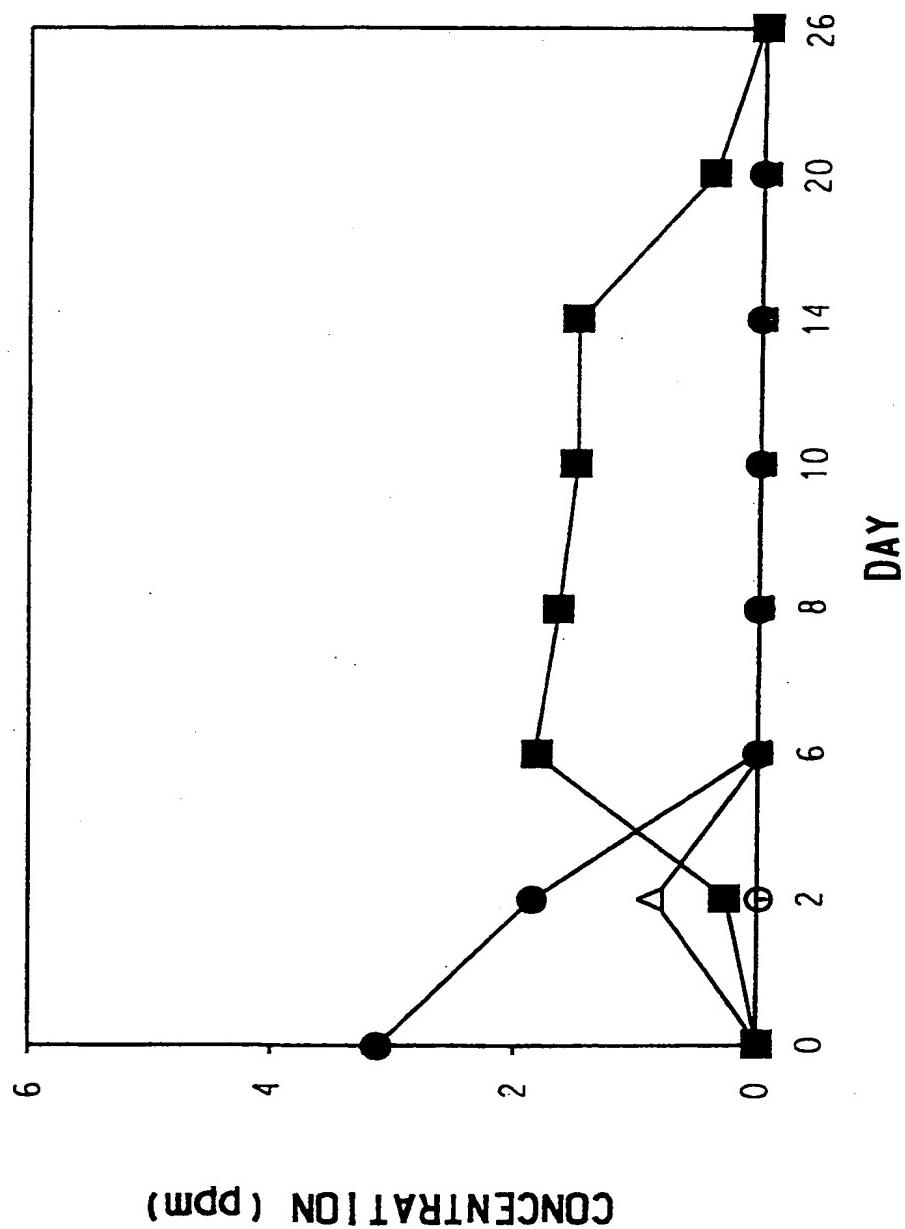
FIG. 2-9

16/17

PCE      TCE      DCE      VC

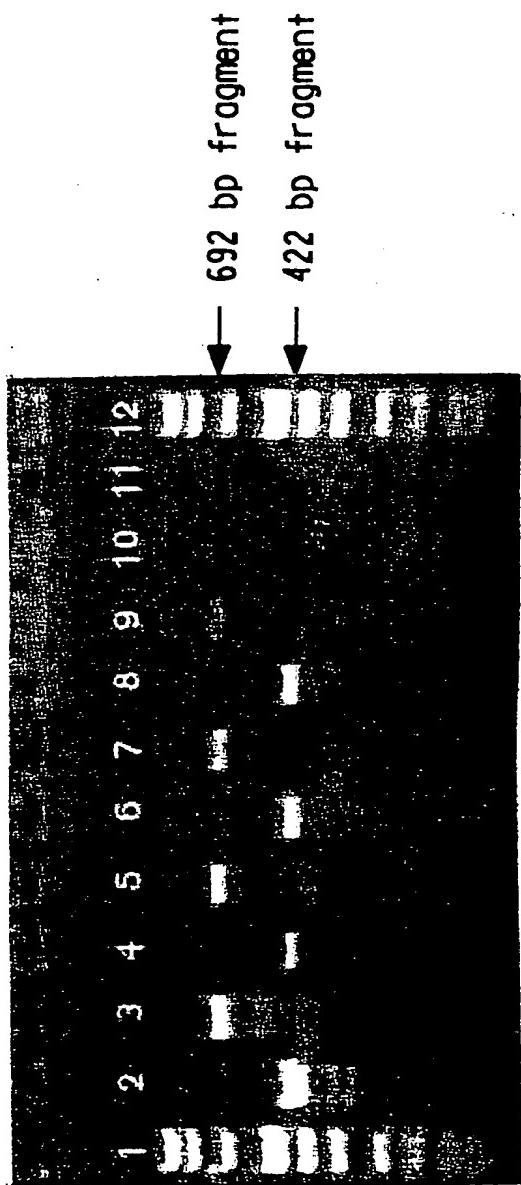
●      △      ■      ○

FIG. 3



17/17

FIG. 4



SEQUENCE LISTING

<110> E. I. DU PONT DE NEMOURS AND COMPANY  
<120> NUCLEIC ACID FRAGMENTS FOR THE IDENTIFICATION OF  
DECHLORINATING BACTERIA  
<130> BC1002 PCT  
<140>  
<141>  
<150> 60/129,511  
<151> 1999-04-15  
<160> 33  
<170> Microsoft Office 97  
<210> 1  
<211> 24  
<212> DNA  
<213> Dehalococcoides ethenogenes

<400> 1  
attttcttagc gagactgccc cgcg

24

<210> 2  
<211> 1212  
<212> DNA  
<213> Dehalococcoides ethenogenes

<400> 2  
gatgaacgct agcggcgtgc cttatgcatttcaagtcgaaac ggtcttaagc aattaagata 60  
gtggcaaaccg ggtgatggtaac gcgttaagttaa cctacccctta agtgggggat agcttcggga 120  
aactgaaggta aataccgcatttgcgtt gacataagtc ggttcattaa agccgcagg 180  
tgcttggtaa ggggcttgcgtt tccgatttagc tagttggggat ggttatggcc taccaaggct 240  
tcgatcggta gctggcttgcgtt gaggatgatc agccacactg ggactgagac acggcccaaga 300  
ctcctacggg aggccggcaggc aaggaaatctt gggcaatggggat cggaaaggctt acccagcaac 360  
gcccggtgagg ggtatggc ttccgggtt taaacccctttt ttcacaggga agaataatga 420  
cggtacccgtt ggaataagct tcggcttaact acgtgcccggc agccggcggtt atacgttagga 480  
agcaaggcgtt atccggatttcccggtt aagtggatcgtt aggtgggtt tcaagttgg 540  
tgtgaaatttcccggtttaa ccgggacgtt tcattcaata ctgttggact agagtacacgc 600  
aggagaaaaac gaaattcccggtt gtgtatggggat aaaatgcgtt gatatggggat ggaacaccag 660  
aggcgaaggc gggttttttagt gtttgcactt acactggc tcgaaaaggctt ggggagccgaa 720  
cagaatttgcgtt tactctggta gtccacggct taaaatggggat acactggat tagggaggtat 780  
cgaccctctt tggccggatccaa ctaacgctt aagtgtccccg cctggggaggt acgggtcgca 840  
ggctaaaact caaaggaaatttgcgtt gacggggggcc cttacaagca gcggagcgtt tggttttaatt 900  
cgatgcata cgaagaacctt tacaaggatt tgacatgcatttgcgtt gaaatggggat accggaaagg 960  
aaacgacccgtt ttaagtgcgtt agtttgcaca ggtgctgcatttgcgtt ggctgtcgcc agctcggtt 1020  
gtgagggttttgcgtt tggtaatgcgtt ctgcaacggat cggcaaccctt gttgttagtt aaattttctt 1080  
gcatggactgc ccccgcaaaac gggggaggaaatgcgtt gttggggatgcgtt cggtaatgcgtt gcatggccctt 1140  
tatatcttggt gctacacacatacgcttacaatgcgtt gacagaacaa taggttgcaatgcgtt gcatggccctt 1200  
tggagctaatcc 1212

<210> 3  
<211> 1335  
<212> DNA  
<213> Dehalococcoides ethenogenes

<400> 3  
gatgaacgct agcggcgtgc cttatgcatttcaagtcgaaac ggtcttaagc aattaagata 60  
gtggcaaaccg ggtgatggtaac gcgttaagttaa cctacccctta agtgggggat agcttcggga 120  
aactgaaggta aataccgcatttgcgtt gacataagtttgcgtt ggttcactaa agccgcagg 180  
tgcttggtaa ggggcttgcgtt tccgatttagc tagttggggat ggttatggcc taccaaggct 240  
tcgatcggta gctggcttgcgtt gaggatgatc agggatgatc cagccacactt gggactgaga cacggcccaaga 300

actcctacgg gaggcagcag caaggaatct tgggcaatgg gcgaaagcct gaccaggcaa 360  
 cgccgcgtga qggatgaagg ctctcggtt gtaaacctct tttcacaggg aagaataatg 420  
 acggtacctg tggaaaataaagc ttccggctaact tacgtgccag cagccgcggg aatacgtagg 480  
 aagcaagcgt tatccggatt tattggcgt aaagtgagcg taggtggctt ttcaagttgg 540  
 atgtgaaatt tcccggctt accgggacgt gtcattcaat actgttggac tagagtagac 600  
 caggagaaaa cggaaattccc ggtgttagtgg taaaatgcgt agatatcggg aggaacaccca 660  
 gaggcgaagg cgggtttcta ggttgtcaact gacactgagg ctcgaaagcg tggggagcga 720  
 acagaattag atactctgtt agtccacgcct ttaaaactatg gacactaagt ataggaggta 780  
 tcgaccctct ctgtggcggaa gctaacgcct taagtgtccc gcctggggag tacggtcgca 840  
 aggctaaaac tcaaaggaaat tgacggggc cgcacaaggc agcggagcgt gtgggttaat 900  
 tcgatctac acgaaaggaaat ttaccaagat ttgacatgc tgaaggtagt aaccgaaaagg 960  
 gaaacgacct gttaaatgtcag gagtttgac aggtgctgca tggctgtcg tgcgtgc 1020  
 cgtgagggtgt ttggtaaagt cctgcaacga gcgcaaccct tggctgtcg taaatttct 1080  
 agcgagactg ccccgcgaaa cggggaggaa ggtggggatg acgtcaagt acgtggcct 1140  
 ttatatcttq ggtacacac acgttacaat ggacagaaca atagggtgca acagtgtgaa 1200  
 ctggagctaa tcctcaaaggc tgcctcgt tcggatttgca ggctgaaacc cgcctgcatt 1260  
 aagttggagt tgcttagtaac cgcatatcgtt caaggtgcgg tgaatacgtt ctcggccct 1320  
 gtacacaccg cccct 1335

<210> 4  
 <211> 1212  
 <212> DNA  
 <213> Dehalococcoides ethenogenes

<400> 4  
 gatgaacgct agcggcgtgc cttatgcattt caagtcgaac ggtcttaaagc aattaagata 60  
 gtggcgaacg ggtgagtaac gcgtaagtaa cctaccccta agtggggat agcttcggga 120  
 aactgaaggt aataccgcattt gttggggcc gacatagtt ggttcaactaa agccgttaagg 180  
 cgcttggta ggggcttgcg tccgatttgc tagttggggat ggtaatggcc taccaggct 240  
 tcgatcggtt gctggcttgcg gaggatgtc agccacactg ggactgagac acggcccaga 300  
 ctccctacggg aggacgcagc aaggaaatctt gggcaatggg cgaaaggctg acccagcaac 360  
 gccgcgttag ggtgaaaggc ttccgggtt gttttttttt ttcataaggaa agaataatga 420  
 cggtacctgtt ggttcaactt acgttgcggc agccgcggta atacgttagga 480  
 agcaagcggtt atccggattt attgggcgtt aagtggcggtt aggtggctt tcaagttgg 540  
 tgtgaaatattt cccggcttaa ccgggacggat ttcattcaata ctgttggact agagtacagc 600  
 aggagaaaaac ggaattcccc ggtgttagtgg tttttttttt ttcataaggaa ggaacaccag 660  
 aggacgaaaggc gtttttctt gttgtcaactt acacttgcggc tccggggatg ggggagcggaa 720  
 cagaatttgcg tactctggta gtccacgcct taaactatgg acacttaggtt tagggagttat 780  
 cgaccctctc tggccgaag ctaacgcctt aagtgtcccg cctggggatg acggtcgca 840  
 ggctaaaactt caaaggaaattt gacggggggcc cgcacaaggc gcggagcgtg tgggttaatt 900  
 cgatgcata cgaaggaaacctt taccaggattt tgacatgc tttttttttt ttcataaggaa actgaaagg 960  
 gaacgacctg ttaagtgcagg aacttgcaca ggtgtcgat ggctgtcg tgcgtgc 1020  
 gtgagggtgtt tggtaaagtgc tgcacacgcg cgcacccctt tggctgtcg tttttttttt ttcataaggaa actgaaagg 1080  
 gcgagactgc ccccgcgaaaac gggggaggaaat gttggggatg cgtcaagtca gcatggcctt 1140  
 ttatatcttq ggtacacacac acgttacaat gacagaacaa taggttgcac tgggtgtgaaac 1200  
 tggagctaat cc 1212

<210> 5  
 <211> 1212  
 <212> DNA  
 <213> Dehalococcoides ethenogenes

<400> 5  
 gatgaacgct agcggcgtgc cttatgcattt caagtcgaac ggtcttaaagc aattaagata 60  
 gtggcggaaacg ggtgagtaac gcgtaagtaa cctaccccta agtggggat agcttcggga 120  
 aactgaaggt aataccgcattt gttggggcc gacatagttt ggttcaactaa agccgttaagg 180  
 tgcttggta ggggcttgcg tccgatttgc tagttggggat ggtaatggcc taccaggct 240  
 tcgatcggtt gctggcttgcg gaggatgtc agccacactg ggactgagac acggcccaga 300  
 ctccctacggg aggacgcagc aaggaaatctt gggcaatggg cgaaaggctg acccagcaac 360  
 gccgcgttag ggtgaaaggc ttccgggtt gttttttttt ttcataaggaa agaataatga 420  
 cggtacctgtt ggttcaactt acgttgcggc agccgcggta atacgttagga 480  
 agcaagcggtt atccggattt attgggcgtt aagtggcggtt aggtggctt tcaagttgg 540  
 tgtgaaatattt cccggcttaa ccgggacgtt ttcattcaata ctgttggact agagtacagc 600  
 aggagaaaaac ggaattcccc ggtgttagtgg tttttttttt ttcataaggaa ggaacaccag 660  
 aggacgaaaggc gtttttctt gttgtcaactt acacttgcggc tccggggatg ggggagcggaa 720  
 cagaatttgcg tactctggta gtccacgcct taaactatgg acacttaggtt tagggagttat 780

cgaccctctc	tgtgccgaag	ctaacgcttt	aagtgtcccg	cctggggagt	acggtcgcaa	840
ggctaaaact	caaaggaaatt	gacgggggccc	cgcacaagca	gcggagcgtg	tggtaatt	900
cgatgctaca	cgaagaacct	taccaagatt	tgacatgcat	gaagtagtga	accgaaagg	960
aaacgacctg	ttaagtcaagg	agttgcaca	ggtgctgcat	ggctgtcgtc	agctcgtgcc	1020
gtgaggtgtt	tggtaagtc	ctgcaacgag	cgcaaccctt	gttgctagtt	aaattttcta	1080
gcgagactgc	ccccgaaaaac	ggggaggaag	gtggggatga	cgtcaagtca	gcatggcctt	1140
tatatcttgg	gctacacacaca	cgctacaatg	gacagaacaa	tagttgcaa	cagtgtgaac	1200
tggagctaat	cc					1212

<210> 6  
<211> 1212  
<212> DNA  
<213> Deha

<400>	6	gatgaacgcgtt	acggcggttc	cttatgcatt	caagtcgaa	ggtcttaagc	aattaagata	60
gtggcaaacg		ggtgagtaac	gcgttaagta	cctacctcta	agtggggat	agttcggga		120
aactgaaggt		aataccgcat	gtgggggcc	gacataagg	ggttcaactaa	agccgtaaagg		180
tgcttggtga		ggggcttgcg	tccgattagc	tagttggtg	ggtaacggcc	taccaaggct		240
tcgatcggtt		gctggtctga	gaggatgatc	agccacactg	ggactgagac	acggccccaga		300
ctccctacggg		aggcagcagc	aaggaatctt	ggcataatggg	cgaaaggctg	accaggcaac		360
gcccgttag		ggatgaaggc	tctcggtt	taaacatctt	ttcacaggga	agaataatgt		420
cggtacctgt		ggaataact	tccgctact	acgtgcca	agcccggtt	atacgttagga		480
agcaagcgtt		atccggattt	attggcgta	aagttagcgt	aggttgttctt	tcaagttgga		540
tgtgaaattt		cccggttaa	ccgggacgtg	tcattcaata	ctgtggact	agagtacagc		600
aggagaaaac		ggaattcccg	gtgttagtgg	aaaatgcgt	qatatcggtt	ggaacaccag		660
aggcgaaggc		gttttctag	gttgcactg	acactgaggc	tcgaaaagcgt	ggggagcga		720
cagaattaga		tactctggta	gtccacgcct	taaactatgg	acacttagta	tagggagtat		780
cgaccctctc		tgtgccgaag	ctaacgcctt	aagtgtcccg	cctggggagt	acggtcgcaa		840
ggctaaaact		caaaggaatt	gacgggggccc	cgcacaagca	gcggagcgtg	tggtttaatt		900
cgtatgtaca		cgaagaacct	taccaagatt	tgacatgcatt	gaagttagtga	accggaaagg		960
aaacgacactg		ttaagtccagg	agtttgcaca	ggtgctgcatt	ggctgtgc	agctcggtcc		1020
gtgaggttgtt		tgttaaagt	ctgcaacgag	cgcaaccctt	gttgctagtt	aaattttctt		1080
gcgagatcgc		cccgcgaaac	ggggaggaaag	gtggggatga	cgtcaagtca	gcatggccctt		1140
tatatcttgg		gctacacaca	cgctacaatg	gacagaacaa	tagttgcaa	cagtgtgaac		1200
tggagctaat		cc						1212

<210> 7  
<211> 1443  
<212> DNA  
<213> Reba

<400>	7					
gatgaacgct	agcggcggtgc	cttatgcatt	caagtcgaac	ggtcttaagc	aattaagata	60
gtggcaaacg	ggtgagtaac	gcgttaagtaa	cctacctcta	agtgggggat	agttcggga	120
aactgaaggt	aataccgcat	gtgatggct	gacataagtc	ggttcattaa	agccgcaagg	180
tgcgttggta	ggggcttgcg	tccgatttagc	tagttggtgg	ggtaatggtc	taccaaggct	240
tcgatcggt	gctggctctga	gaggatgatc	agccacactg	ggactgagac	acgggccaga	300
ctccctacgg	aggcagcgc	aaggaatctt	gggcaatggg	cgaaagcctg	acccagcaac	360
gcccgcgtgg	ggatgaaggc	tttcgggtt	taaaccttctt	ttcacaggga	agaataatga	420
cggtacactgt	ggaataagct	tccgttaact	acgtgcccgc	acggccggta	atacgttggg	480
aagcaagcgt	tatccggatt	tattgggcgt	aaagtggacg	taggtggtct	ttcaagttgg	540
atgtgaaatt	tcccggttta	accgggacgt	gtcattcaat	actgtggac	tagatcag	600
caggagaaaa	cgaattccc	ggtgttagtgg	taaaaatgggt	agatatcggt	aggaacacca	660
gaggcgaagg	cgttttctt	ggttgtcaact	gacactgagg	ctcgaaagcg	tggggagcga	720
acagaattag	atactctggt	agtccacgccc	ttaaactatg	gacacttaggt	atagggagta	780
tcgaccctct	ctgtggcgaa	gctaacgctt	taagtgtccc	gcctggggag	tacggtcgca	840
aggctaaaac	tcaaaggaat	tgacgggggc	ccgcacaagc	agcggagcgt	gtggtttaat	900
tcgatgtac	acgaagaact	taccaagatt	tgacatgtat	gaagtagtga	accgaaagg	960
aaacgacctg	ttaagtgcagg	agtttgcaca	ggtgctgcatt	ggctgtcgcc	agctcggtgc	1020
gtgagggtgtt	gggttaagtc	ctgcaacgc	cgcaacccgt	ttgtcttagtta	aattttctag	1080
cgagactagc	gagactgccc	cgcgaaacgg	ggaggaaggt	ggggatgacg	tcaagtgc	1140
atggccctta	tatcttgggc	tacacacacg	ctacataatgg	cagaacaata	ggttgcaca	1200
gtgtgaactg	gagctaattcc	ccaaagctgt	cctcagttcg	gattgcaggc	tggaaaccgc	1260
ctgcatgaag	tttgagttgc	tagtaaccgc	atatcagcaa	ggtgccgtga	atacgttctc	1320
gggccttgc	cacaccgcccc	gtcacgtcat	ganagccgg	aacacttggaa	gtcgatgtqc	1380

caaccgcaag gaggcagtgc ccgagggtgg gactggtaat tgggacgaag tcgtaacaag 1440  
gta 1443

<210> 8  
<211> 47  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: CONSENSUS

<220>  
<221> unsure  
<222> (5)  
<223> R=A/G

<220>  
<221> unsure  
<222> (11)  
<223> Y=C/T

<220>  
<221> unsure  
<222> (18)  
<223> W=A/T

<220>  
<221> unsure  
<222> (21)  
<223> Y=C/T

<220>  
<221> unsure  
<222> (28)  
<223> Y=T/C

<220>  
<221> unsure  
<222> (37)  
<223> Y=T/C

<220>  
<221> unsure  
<222> (42)  
<223> Y=C/T

<400> 8  
tgtgrtgggc ygacatawgt yggttcayta aagccgyaag gygcgg 47

<210> 9  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 9  
aagtcgaacg gtcttaagca 20

<210> 10  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 10  
cgtcattatt cttccctgtg 20

<210> 11  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 11  
gggaaacgac ctgttaagtc a 21

<210> 12  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 12  
ggatttagctc cagttcacac tg 22

<210> 13  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 13  
aaatttaact agcaacaagg 20

<210> 14  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 14  
ggagtatcg ccctctctg 19

<210> 15  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 15  
gggagtatcg accctctc 18

<210> 16  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 16  
agtgaaccga aaggaaaa 18

<210> 17  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 17  
gggttgtaaa cctctttca c 21

<210> 18  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 18  
gttagcttcg gcacagagag 20

<210> 19  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 19  
tcagtgacaa cctagaaaac 20

<210> 20  
<211> 17  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 20  
gatgaacgct agcggcg 17

<210> 21  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 21  
gtgccttatg catgcaag 18

<210> 22  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 22  
aatagggttgc aacagtgtga a 21  
  
<210> 23  
<211> 22  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 23  
aatggacaga acaataggtt gc 22  
  
<210> 24  
<211> 21  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 24  
ggcacatcga cttcaagtgt t 21  
  
<210> 25  
<211> 21  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 25  
ggcacatcga cttcaagtgt t 21  
  
<210> 26  
<211> 20  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 26  
taaccgggac gngtcattca 20  
  
<210> 27  
<211> 19  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PRIMER  
  
<400> 27  
gagtagcagca ggagaaaaac 19  
  
<210> 28  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 28  
cctccttgcg gttggcacat c 21

<210> 29  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PRIMER

<400> 29  
ggcagtctcg ctagaaaaat 19

<210> 30  
<211> 51  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: CONSENSUS

<220>  
<221> unsure  
<222> (3)  
<223> W=A/T

<220>  
<221> unsure  
<222> (14)  
<223> M=A/C

<220>  
<221> unsure  
<222> (22)  
<223> R=A/G

<220>  
<221> unsure  
<222> (43)  
<223> R=A/G

<220>  
<221> unsure  
<222> (44)  
<223> M=A/C

<400> 30  
tgwagttagtg aacmgaaagg graacgacct gttaagtcag garmttgcac a 51

<210> 31  
<211> 18  
<212> DNA  
<213> Dehalococcoides ethenogenes

<400> 31  
attttctacg cgagactg 18

<210> 32  
<211> 27  
<212> DNA  
<213> Dehalococcoides ethenogenes

<400> 32  
attttctacg cgagacttagc gagactg

27

<210> 33  
<211> 1542  
<212> DNA  
<213> E. COLI

<400> 33  
aaattgaaga gtttgatcat ggctcagatt gaacgctggc ggcaggccata acacatgcaa 60  
gtcgaacggt aacaggaaga agcttgcttc tttgctgacg agtggcggac gggtagtaa 120  
tgtctggaa actgcctgat ggagggggat aactactgaa aacggttagct aataccggcat 180  
aacgtcgcaa gaccaaagag ggggaccttc gggcctctg ccatcgatg tgcccagatg 240  
ggattagcta gttagtgggg taacgctca cctaggcgac gatcccttagc tggcttgaga 300  
ggatgaccag ccacactgga actgagacac gttccagact cctacgggag gcagcagtgg 360  
ggaatattgc acaatggcg caagcctgat gcagccatgc cgctgtatg aagaaggcct 420  
tcgggttgta aagtactttc agcggggagg aagggagtaa agttaataacc ttgtctcatt 480  
gacgttaccc gcagaagaag caccggctaa ctccgtgcca gcagccgccc taatacggag 540  
gttgcaagcg ttaatcgaa ttactggcg taaagcgcac gcaggcggtt ttgttaagtca 600  
gatgtgaaaat ccccgggctc aacctggaa ctgcattctg tactggcaag ttgtgatctc 660  
gttagaggggg gtagaatttcc aggtttagcg gtgaaatgcg tagagatctg gaggaaatacc 720  
ggtggcgaag gccccccctt ggacaaagac tgacgctca gtcgacttgcgatg 780  
aacaggattt gatccccctgg tagtcacacgc ctgtaaacatgat gtcgacttgg aggttgc 840  
cttgaggcggtt ggcttccggaa gctaacgcgt taagtcgacc gcctggggag tacggccca 900  
aggttaaaac tcaaataaatgat tgacgggggc cccgcacaaagc ggtggagcat gtggtttaat 960  
tcgatgcaac gccaagaacc ttacctggtc ttgacatccca cggaaatggc cagagatgag 1020  
aatgtgcctt cgggaaccgt gagacagggtg ctgcattggct gtcgacttgcgatg 1080  
aatgttgggt taagtcccgc aacgagcgc acccttatcc tttgttgcca gcgggtccggc 1140  
cgggaaactca aaggagactg ccagtataa actggaggaa ggtggggatg acgtcaagtc 1200  
atcatggccc ttacgaccag ggctacacac gtgttacaat ggcgcataaca aagagaagcg 1260  
acctcgcgag agcaagcggc cctcataaaag tgcgtcgtag tccggattgg agtctgcaac 1320  
tcgactccat gaagtggaa tcgttgcgttca gaatgccacg gtgaataacgt 1380  
tccccgggcct ttacacacc gcccgtcaca ccatggggat ggttgcaaaa agaagtaggt 1440  
agcttaacct tcggggggc gtttaccat ttgtgattca tgactggggt gaagtcgtaa 1500  
caaggtaacc gttagggaaac ctgcgttgg atcacctcct ta 1542

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
26 October 2000 (26.10.2000)

PCT

(10) International Publication Number  
**WO 00/63443 A3**

(51) International Patent Classification<sup>7</sup>: C12Q 1/68.  
A62D 3/00

19707 (US). EBERSOLE, Richard, C. [US/US]; 2412  
Dacia Drive, Wilmington, DE 19810 (US).

(21) International Application Number: PCT/US00/09883

(74) Agent: FELTHAM, S., Neil: E.I. du Pont de Nemours  
and Company, Legal Patent Records Center, 1007 Market  
Street, Wilmington, DE 19898 (US).

(22) International Filing Date: 13 April 2000 (13.04.2000)

(81) Designated States (national): CA, MX, US.

(30) Priority Data:

60/129,511 15 April 1999 (15.04.1999) US

Published:  
— with international search report

(71) Applicant (for all designated States except US): E.I. DU  
PONT DE NEMOURS AND COMPANY [US/US]; 1007  
Market Street, Wilmington, DE 19898 (US).

(88) Date of publication of the international search report:  
10 May 2002

(72) Inventors; and

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(75) Inventors/Applicants (for US only): HENDRICKSON,  
Edwin, R. [US/US]; 49 Kings Grant Road, Hockessin, DE



WO 00/63443 A3

(54) Title: NUCLEIC ACID FRAGMENTS FOR THE IDENTIFICATION OF DECHLORINATING BACTERIA

(57) Abstract: A unique 16S rRNA profile derived from *Dehalococcoides ethenogenes* has been identified and isolated. The profile contains a nucleic acid fragment that is linked to dechlorinating activity. This sequence is set forth in SEQ ID NO:1.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/09883

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12Q1/68 A62D3/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12Q A62D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name or data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, EMBASE, MEDLINE, BIOSIS, SEQUENCE SEARCH

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MAYMO-GATELL XAVIER ET AL: "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." SCIENCE (WASHINGTON D C), vol. 276, no. 5318, 1997, pages 1568-1571, XP002179676 ISSN: 0036-8075 the whole document &amp; DATABASE EMBL:DEAF4928 'Online! EMBL; 4 July 1997 (1997-07-04) MAYMO-GATELL XAVIER ET AL: retrieved from EMBL, accession no. AF004928 Database accession no. EMBL:DEAF4928 abstract</p> <p>---</p> <p style="text-align: center;">-/-</p>	1-9

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents:

- \*'A' document defining the general state of the art which is not considered to be of particular relevance
- \*'E' earlier document but published on or after the international filing date
- \*'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*'O' document referring to an oral disclosure, use, exhibition or other means
- \*'P' document published prior to the international filing date but later than the priority date claimed

- \*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*'Z' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 2001	23/10/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer  Gabriels, J

## INTERNATIONAL SEARCH REPORT

International Application No PCT/US 00/09883
---

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation or document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HOLOMAN TRACEY R PULLIAM ET AL:          "Characterization of a defined          2,3,5,6-tetrachlorobiphenyl-ortho-dechlorinating microbial community by          comparative sequence analysis of genes          coding for 16S rRNA."  <i>APPLIED AND ENVIRONMENTAL MICROBIOLOGY</i>,          vol. 64, no. 9, 1998, pages 3359-3367,          XP002179677          ISSN: 0099-2240          page 3364 -page 3366, left-hand column;          table 2</p> <p>---</p>	1-9
X	<p>VON WINTZINGERODE FRIEDRICH ET AL:          "Phylogenetic analysis of an anaerobic,          trichlorobenzene-transforming microbial          consortium."  <i>APPLIED AND ENVIRONMENTAL MICROBIOLOGY</i>,          vol. 65, no. 1, January 1999 (1999-01),          pages 283-286, XP002179679          ISSN: 0099-2240          page 284, right-hand column -page 286,          left-hand column</p> <p>---</p>	1-9
X	<p>LAMONTAGNE M G ET AL: "Identification and          analysis of PCB dechlorinating anaerobic          enrichments by amplification: Accuracy of          community structure based on restriction          analysis and partial sequencing of 16S          rRNA genes."  <i>JOURNAL OF APPLIED MICROBIOLOGY</i>,          vol. 84, no. 6, June 1998 (1998-06), pages          1156-1162, XP001024984          ISSN: 1364-5072          page 1157, right-hand column -page 1161,          right-hand column</p> <p>---</p>	1-9
X	<p>FR 2 733 754 A (UNIV ANGERS)          8 November 1996 (1996-11-08)          SEQ 3 of FR2733754 hybridises specifically          with SEQ ID NO:2 of the present          application          example 1</p> <p>---</p>	1,3
X	<p>WO 98 49106 A (UNIV IOWA RES FOUND ; PARKIN          GENE F (US); ALVAREZ PEDRO J (US); TIL)          5 November 1998 (1998-11-05)</p>	8,9
A	Claims 1-3, 42-45	1-9
A	<p>EP 0 864 542 A (SOLVAY DEUTSCHLAND)          16 September 1998 (1998-09-16)</p>	1-9
	example 5	
	---	
	-/-	

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/09883

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 540 838 A (SMULLEN LYNN A ET AL) 30 July 1996 (1996-07-30) Claim 1 -----	1-9

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/09883

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
FR 2733754	A	08-11-1996	FR	2733754 A1		08-11-1996
WO 9849106	A	05-11-1998	AU WO	7152298 A 9849106 A1		24-11-1998 05-11-1998
EP 0864542	A	16-09-1998	DE EP	19710010 A1 0864542 A2		17-09-1998 16-09-1998
US 5540838	A	30-07-1996		NONE		